Toxicology and Potential Health Risk of Chemicals that May Be Encountered by Workers Using Forest Vegetation Management Options

PART III: RISK TO WORKERS USING 2,4-D FORMULATIONS

Forest Practices Branch
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Abstract

2,4-D is possibly the most extensively researched of all pesticides, and the data have been examined by an unusual number of advisory committees and work groups.

2,4-D is slowly absorbed from the skin, and is rapidly excreted unchanged by the kidneys. It is not stored in the body. Mutagenic activity of 2,4-D is negligible or absent, nor is there evidence of carcinogenicity in animal assays. It does not cause significant reproductive effects except at doses high enough to cause general intoxication. Its ability to cause birth defects is very limited.

The large number of epidemiology studies seeking evidence of a relation between phenoxy herbicides and human cancer has been inconsistent and conflicting. Review panels, including that convened by United States Environmental Protection Agency (USEPA) in April 1993 have consistently concluded that the evidence is at best weakly suggestive and does not warrant change in regulatory policy.

A number of cases of human intoxication from either careless handling or suicide attempts have been reported in the medical literature, almost all in the fifties and sixties. The pattern of effects has been inconsistent but a few individuals have experienced neurologic problems in the extremities.

There is extensive data on exposure of forest workers to 2,4-D, showing that careless work habits increase exposure. The primary concern is skin and eye irritation from certain formulations. Simple protective clothing and work discipline reduce exposure to very low levels.

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Foreword

Vegetation management is an important reforestation activity for controlling competing vegetation or brush encroachment of young tree seedlings. The activity is necessary to get tree seedlings to free-growing status in most new forest sites established in areas that have been harvested or denuded by wildfire, insects and disease.

There are a number of options for managing forest vegetation. The treatment options include prescribed fire, herbicides, manual removal with hand and power tools (e.g., girdling and slashing tools, chain saws and brush saws), placement of mulch mats, mechanical techniques with heavy machinery, and biological methods. The use of livestock (e.g., sheep) is currently the common biological control technique employed in reforestation areas in British Columbia. Biological methods with insects or specific pathogens is used on forest rangelands for noxious weed control but not commonly used for vegetation control in young forest stands.

The selection of a treatment option involves a decision-making process based on integrated vegetation management concepts that include evaluation of the need for treatment, consideration of all the approved treatment methods and choosing the most appropriate treatment method, monitoring and evaluation. Factors considered in selecting a particular method are the ability of the method to meet the required reforestation objectives, the impact of the treatment at the specific site on human safety and the environment (e.g., recreational resources, fish and wildlife and their habitat, range resources and water supply), as well as the economics of the treatment.

This publication is one of a series of papers that evaluates the potential health effects on forest workers using the commonly employed methods of vegetation control. Other papers in the series are listed at the end of this paper. The emphasis is on risks associated with exposure to chemicals during the use of two most important methods for controlling competing vegetation in regenerated (natural or planted) forest areas. These methods are the use of herbicides and manual removal or control with handheld-motorized (power) equipment.

The herbicides discussed are those that have been commonly used in forestry in Canada. The database on health effects of herbicides is extensive and permits reliable estimates of risk. For components of chain saw exhaust and fuels, there is also voluminous background of toxicological information, but exposure data in forestry is limited. Nonetheless, there is enough information to develop preliminary assessments of potential health effects. While there appears to be a high incidence of physical injury associated with manual methods of brush control, there is virtually no validated data on which to base estimates of risk. The existing data are those of workers compensation boards and insurance companies but such data are generally difficult to obtain or are not specifically enough to characterize the kind of activity that leads to injury.

The information in these reports should provide the basis for important decisions about the way vegetation management in forestry should be carried out, and the use of some forestry activities as a source of assisted employment.

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Introduction

2,4-D was first registered in 1947 as a broadleaf herbicide for use in all facets of vegetation management. At the time it first became available, toxicological and environmental assessment was minimal. As more stringent registration requirements were established more and more data were added. Academic and governmental laboratory and epidemiologic research has also been prolific because any substance of such wide use draws both basic and field research. In addition, 2,4-D was a component of Agent Orange, which became the dominant social symbol of the Vietnam War. In that context, it acquired a mystique about health effects that was independent of real information. A great deal of research has been directed at clarifying those perceptions. Because of the widespread research attention, 2,4-D has a more extensive scientific base than any other herbicide, and perhaps any other pesticide.

In addition, because of both scientific and social concerns about 2,4-D, various advisory groups and workshops have been assembled over time to examine and interpret the data.

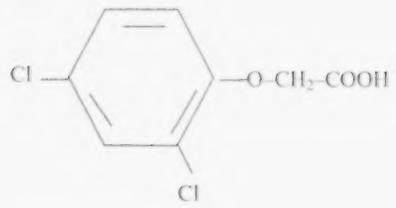
This discussion is organized in two stages. The earlier research is summarized largely from several review documents prepared in the mid-eighties, the most useful of which were those of the US Forest Service (USDA-Forest Service, 1984); Newton and Dost (1984) and Shipp et al (1986). The latter were both prepared for the Washington State Department of Natural Resources. Where necessary, pertinent primary references are discussed. The second stage is a review of more recent literature from the mid-eighties to the present. The section on the possible carcinogenicity of 2,4-D includes discussion of the findings of several expert panels assembled to examine the experimental and epidemiological data. Selected epidemiological findings are also discussed specifically to illustrate the difficulties in deriving meaningful conclusions from these studies.

There are several published reviews of the toxicology of 2,4-D. That of Munro et al (1992) occupies an entire issue of the Journal of the American College of Toxicology. Recent reviews have also been prepared by Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) (FAO-WHO, 1996) and by United States Environmental Protection Agency (USEPA, 1999).

Forms of 2,4-D

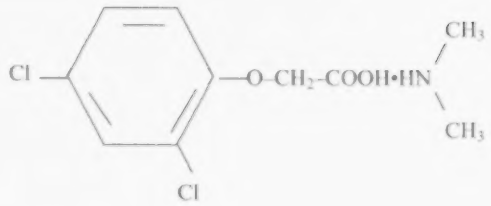
Commercial 2,4-D products are usually amine salts or esters of the parent acid. The dimethyl-amine salt is highly soluble in water, soluble in alcohols and insoluble in hydrocarbons like diesel oil and kerosene. The low volatile butoxyethanol ester of 2,4-D is perhaps the most common of that family. Another example of a low volatile ester of the chemical is iso-octyl ester of 2,4-D. The esters are insoluble in water and soluble in kerosene and similar solvents. Over the long history of 2,4-D there have been several variants of these attached groups. Some highly volatile esters have been used in the past in agriculture, but tend to move into the atmosphere, occasionally causing crop damage downwind.

Structural Formula of 2, 4-D acid

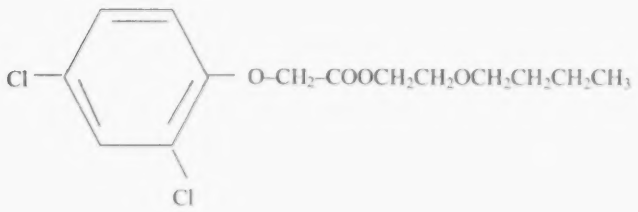


(Molecular Formula: $C_8H_6Cl_2O_3$)

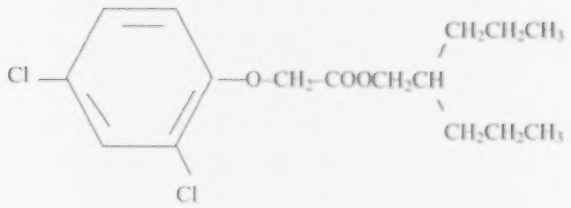
Structural Formula of dimethylamine salt of 2,4-D



Structural Formula of Butoxyethanol ester of 2,4-D (a low volatile ester)



Structural Formula of iso-octyl ester of 2,4-D (a low volatile ester)



Behaviour of 2,4-D in the Body: Absorption, Metabolism and Excretion

Absorption from body surfaces

To produce a biological effect, a chemical either reacts at the surface of first contact, such as the skin, respiratory or digestive tracts, or it must find its way into the circulation to be distributed throughout the body to the various organs and structures. It must then either interact at the surface of cells or enter cells to alter some constituent that is important to normal function of the organism. The longer it remains in the body, the greater the potential for harmful effect.

2,4-D is not readily absorbed from most areas of the skin but the rate of absorption varies with body area and duration of contact. Feldman and Maibach (1974) observed that 5.6% of radiolabelled 2,4-D was absorbed from the forearm of human volunteers over a 24 hour period. Apparently 2,4-D acid was used, although this was not specifically stated. The herbicide was applied in an acetone solution, and protected by a bandage during the study period. Both treatments would accelerate absorption across the skin. This absorption rate has been accepted as the standard in risk assessments even though it appears not to be representative of field exposure or proper work hygiene. Recent work provides more useful estimates.

Moody et al (1990) compared absorption rates of 2,4-D amine in water and the isooctyl ester in acetone from various body areas in different species. It was not possible to test all preparations on all sites, however, so some comparisons were not complete. The test substance was left in place for 24 hours, then washed off, and urine was collected for seven days. One remarkable finding was that 2,4-D amine in water was absorbed to the extent of 58% from the human forehead. After the amine was applied to skin on the back of rabbits, 12% of the material applied to the back of the rabbit was absorbed over 24 hours. In isolation, the differences could be attributed to unknown species and site differences. However, in contrast, the isooctyl ester in acetone was absorbed to the extent of 50% across the skin of the back of the rabbit and only 6% from the skin of the human forehead. There were inconsistencies in the findings that were not resolved, however. One would expect some difference with formulation and solvent on a given site, but the species/site relationship should remain at least qualitatively similar. The findings are also not consistent with other work.

As might be expected, the period of exposure has great influence on the extent of absorption. Wester and Maibach (1985) include data showing that washing with soap and water within 15 minutes of exposure reduced 2,4-D absorption to about 12% of the amount absorbed when the application was left in place over 24 hours.

Other studies suggest that in the field, absorption of 2,4-D is much lower than the measurements discussed above. Frank et al (1985) described an experiment in which one person wearing just shorts and a short-sleeved shirt, and two subjects wearing full protective gear were directly sprayed purposely with the isooctyl ester of 2,4-D by aircraft. Comparison of deposition on the full suits with the excretion by the scantily clothed subject indicated whole body absorption and excretion of about 0.5% of total deposition. If it is assumed that 65% of the body was protected (Even light clothing will intercept the herbicide if not saturated.) the absorption rate from exposed surfaces would be on the order of 1.5%.

Experiments with compounds that should have characteristics similar to those of 2,4-D indicate similar absorption rates. Chester and Hart (1986) compared deposition of fluazifop-butyl on fully clad workers with urinary excretion by workers with bare legs and arms who were similarly exposed and showered eight hours after exposure. About 1% of estimated skin exposure was absorbed. Fluazifop-butyl has similar lipid and water solubilities to those of 2,4-D isooctyl ester and is excreted essentially unchanged, and should behave similarly to 2,4-D. Carmichael et al (1989) tested absorption of triclopyr ester through the skin of the forearm in laboratory studies and found absorption to be about 2% of the amount applied.

If ingested, 2,4-D is absorbed almost completely from the digestive tract. It should be expected that the ester formulations, which are somewhat more fat soluble, should move across membranes more easily than the water soluble salts, although this has not always proven to be the case.

Metabolism

Once absorbed into the circulation, the various salts and esters of 2,4-D hydrolyze to the parent 2,4-D, which moves freely into most tissues. As soon as intake stops, 2,4-D moves out of tissues to the kidneys and is excreted unchanged within a short time. This behaviour has been studied in humans (Kohli et al, 1974; Sauerhoff et al, 1977) as well as experimental animals. By 48 hours most of a single moderate dose of 2,4-D will have appeared in the urine. 2,4-D is not retained in fat or other tissues and does not biomagnify within food chains. Like many substances transported in the circulation, 2,4-D is bound to serum protein, in this case albumin. The binding is quite strong in thermodynamic terms (Rosso et al, 1998) but the equilibrium between binding and release allows relatively free movement between compartments, as reflected in the rapid removal from tissues, and excretion.

2,4-D requires no change in the body in preparation for excretion, although small amounts are sometimes conjugated with (attached to) an amino acid or other small soluble molecule to facilitate excretion, but the structure is not altered. (Schulze et al, 1985; Kelley and Vessey, 1987; Pelletier et al, 1989). Many chemicals, particularly those that are not easily water soluble must be metabolized (changed) by enzymatic reactions in the liver and other organs to make them soluble to facilitate removal through the kidney or in bile. Some chemicals are partly oxidized to carbon dioxide, which is removed through the lungs. 2,4-D does not require such change.

Excretion of 2,4-D

It has been known for many years that 2,4-D is excreted quickly and essentially unchanged by an active transport system in the tubules of the kidney that is also responsible for excretion of organic acids produced in the body. Important examples of such endogenous organic acids are the metabolites of neurotransmitter substances in the central nervous system, which must be excreted first from the brain to the blood, then from the body through the kidneys. This system, which might be visualized as a "pump," is saturable, with a finite capacity. Because all organic acids presented to this system are

handled alike, large amounts of 2,4-D would occupy capacity that might be needed for excretion of normally occurring substances. While the excretion mechanism is very efficient in most species, including humans, it does not have high capacity in dogs, which accounts for the greater sensitivity of dogs to 2,4-D and similar compounds. This mechanism has been well described in several reports, including Dybing and Kolberg (1967), Berndt and Koschier (1973), Erne and Sperber (1974), and Koschier et al (1978).

The half-time for excretion in the human is on the order of 20 hours or less (Kohli et al, 1974; Sauerhoff et al, 1977) (In a system with a half-time of 20 hours, half of the amount remaining at any given time will be removed in 20 hours. Of the half remaining at 20 hours, half would be gone at 40 hours, leaving 1/4, and so on.) Rapid excretion by humans has been shown at very high doses as well. Young and Haley (1977) observed the course of excretion from an attempted suicide who had ingested about 250 mg 2,4-D/kg body weight and found a half time of about 17 hours, similar to that found in studies of humans ingesting 5 mg/kg.

Systemic Toxicity of 2,4-D: Effects Other Than Reproduction, Genetic Toxicity and Carcinogenesis

Human intoxication

There have been several clinical case reports of human intoxication by 2,4-D. Most occurred in the 1950s and early sixties, when users were often quite careless. All of the human cases apparently involved heavy but undetermined exposures to concentrated formulation, either by ingestion or spillage on skin without washing. Most of these have been reviewed in Newton and Dost (1984). Many of the ingestion cases were suicide attempts. Considering the extensive use of this material over almost 50 years, the number of reports of accidental intoxication is quite small, but they and the suicide reports provide valuable information. There is not a characteristic pattern of symptoms across these cases, but certain responses have been seen in enough exposed individuals to warrant tentative identification with 2,4-D and stimulate more detailed experimental study.

Particularly, in several cases a neuromuscular problem sometimes called peripheral neuropathy has occurred after ingestion or dermal exposure to concentrated material. This response was characterized by numbness, aching of the extremities, muscle twitching and decreased speed of nerve impulse conduction. In certain individuals this condition has persisted for months or years, but in other heavy exposures, including some suicide attempts, no peripheral neurological symptoms have been seen. There have been a number of anecdotal reports of peripheral neuropathy after lesser exposures, but none have been sufficiently documented to evaluate. The absence of a consistent pattern of effects, and the apparent presence of other, confounding conditions raises questions about the connection of the described neurological symptoms with 2,4-D exposure. Particularly, it is curious that these effects arose long after the 2,4-D would have been excreted from the body. The question of neurological injury has been examined in various ways more recently and is discussed again later.

2,4-D, particularly the concentrated formulations, can cause irritation of the skin and eyes. If ingested, gastrointestinal irritation is likely. Prolonged dermal exposure has also been reported to cause gastroenteritis. Central depression has been described after large doses; many organic chemicals produce such effects, and in the case of 2,4-D they are probably non-specific.

In the early 1960s several individuals were given high doses of 2,4-D in attempts at therapy of hopeless cases of cancer or other disease. Their responses are discussed later. The "Ranch

Hands," military personnel who handled and applied Agent Orange, which included 2,4-D, have been followed in a prospective epidemiological study for many years. Their exposures for the tour of duty have been calculated to be at least 1000 times greater than that of an unclothed person directly in a typical spray swath, which is also very high relative to typical workplace exposure. Their clinical findings are also discussed later under effects on specific organ systems.

General systemic effects

2,4-D cannot be said to have a specific mechanism of effect in mammals, but like all chemicals, if the dose can be raised high enough, toxicity will be manifested in some system. The reason that 2,4-D is very toxic to plants is because it mimics a specific plant growth regulator and disrupts plant growth control. No related mechanism exists in mammals, and mammalian effects only occur when the organism has become heavily burdened with the chemical, resulting in non-specific changes. The extensive use of 2,4-D and the social concerns surrounding it have been catalysts leading to numerous studies seeking evidence of more subtle effects on biochemical mechanisms that might have significance in case of long term or excessive exposure. Thus far, there is little evidence of any specific effects of 2,4-D on any sensitive system in mammals.

In addition to academic and industrial research studies, registration data has been upgraded to meet current requirements. Some of this work has been published in peer-reviewed literature. Charles et al (1996a) reported studies comparing chronic and subchronic dietary toxicity of 2,4-D acid, amine and ester in the dog, and subchronic toxicity in the rat (Charles et al, 1996b). Many of the newer tests might be considered as repetition of earlier work, but they incorporate more modern procedures and auditing, and are more informative and reliable.

2,4-D is classified as moderately toxic. Median lethal doses (LD_{50}s) are in the range of 250–400 mg/kg, although the guinea pig is relatively insensitive at about 1000 mg/kg and the dog is the most sensitive at about 100 mg/kg. It is unclear why the guinea pig is less sensitive, but the sensitivity of the dog is due to its uniquely limited capacity for excretion of organic acids.

Gorzinski et al (1987) reported data from single dose experiments and 13-week treatments of rats at daily doses of 15, 60, 100, and 150 mg/kg. They also re-examined the behaviour of 2,4-D in the body after oral administration. The acute tests with 2,4-D acid, the sodium and dimethylamine salts, and isooctyl, isobutyl, butoxyethanol and butyl esters indicated median lethal doses (LD_{50}) on an acid equivalent basis of 536–754 mg/kg for males and 424–840 for females. The reported ranges indicate somewhat lower toxicity than older data, but fall in the same general realm.

At the higher doses a limited increase in liver weight, with cellular swelling, was observed. Thyroxine (thyroid hormone) levels increased slightly at the lower dose in both male and female rats, but at the two highest doses decreased sharply in females, with a lesser decrease in males. The pattern of 2,4-D excretion at various doses indicated saturation of the kidney excretory mechanism for organic acids at any doses over 50 mg/kg. Over all, the authors concluded that the no observed effect level is below 15 mg/kg/day, which is consistent with other observations. With respect to thyroid effects, Van den Berg et al (1991) found a moderate reduction in thyroid hormone levels, but not in levels of the precursor triiodo-thyronine, at a single dose of about 13 mg/kg. This is consistent with observations of Kobal et al (2000) on tri-iodothyronine and thyroxin (thyroid hormones) that treatment with 2,4-D over a 13-day period resulted in decreased levels at doses of 110 mg/kg/day, but little effect at 11 mg/kg/day.

Small haemorrhages in the intestine and lungs have been observed after very high doses of some phenoxy herbicides, including 2,4-D.

Possibly related to this, clofibrate, a drug that has some chemical similarity to phenoxy acids and was used at one time to lower blood lipid (fat) levels, has been found to inhibit the ability of platelets to clump together to form the matrix in which blood clots form. (Platelets are very small cell-like bodies released from cells in the bone marrow. They do not have a nucleus or genetic material.) Elo et al (1991) examined the effect of 2,4-D on the ability of human platelets to aggregate *in vitro*, and found a dose-related effect. The no-effect concentration was about 1.0 mg/ml of medium. In mammalian tissues an equivalent dose would be on the order of 1000 mg/kg. In later work (Ylitalo et al, 1991) concentrations as low as 20 mg 2,4-D/litre were reported to cause slight inhibition of platelet aggregation caused by other chemicals.

It appears that the organ most sensitive to long term exposure to 2,4-D is the kidney. Changes in the kidney are consistent with its role in excreting 2,4-D over the long-term intake of the experiments. When blood levels exceed the capacity for excretion, concentrations in kidney become higher than in any other parts of the body, and it is at this stage that effects on the kidney can be expected. Cellular change in the kidneys appears to be consistent at doses of 60 mg/kg and above, with some evidence of effect at lower doses (Gorzinski 1987). Because effects have been observed in rodents at a lifetime daily dose of 5 mg/kg/day over a lifetime the no-observed-adverse effect level (NOAEL) is set at one mg/kg/day.

Low doses (less than 3 mg/kg) have been reported to have a diuretic effect in male rats, while producing no evidence of adverse effect in the kidney (Knopp and Schiller, 1992)

A diuretic effect at low doses implies some kind of specific mechanism of effect within the kidney, without injury to cells of the kidney. Increased urine flow was seen by Lukowicz-Ratajczac and Krechniac (1988), but at doses of 100 and 150 mg/kg every other day for 12 weeks, which approaches lethal levels. At a dose rate of 100 mg every other day, filtration through the glomerulus of the kidney (the site of initial separation of fluid from blood cells) was not changed, so it is likely that the increased flow in the high dose experiments was a function of significant damage to the kidney tubules resulting in poor reabsorption of fluid back into the bloodstream.

The best experimental evaluation of general toxicity has arisen from the clinical testing of the rats and mice on the carcinogenesis studies reported to USEPA in 1986. Dose rates were 0, 1, 5, 15 and 45 mg/kg/day. In both species, haematology, urinalysis and clinical chemistry measurements were made prior to the study and at 26, 52 and 78 weeks. As expected, there were dose-related changes in kidney structure, occurring at all but the lowest dose rate in rats and in the two higher dose rates in mice. There were no other observable adverse effects found except for decreased weight gain in female rats at the highest dose rate.

Examination of the 995 Ranch Hands and their 1299 controls continues, with emphasis on various end points. The primary concern during the latter years has been association with tissue and plasma levels of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which was a contaminant of 2,4,5-T in Agent Orange. The removal of TCDD from the body is very slow, but the rate is known, permitting its use as a marker for exposure to 2,4-D and 2,4,5-T at the time of the conflict.

The results of the 1987 examinations were presented in 1990 by Wolfe et al. A slight increase in chronic liver disease was detected, but no excess of kidney or thyroid disease. Percentages of abnormals among the several kinds of liver function tests were similar in both groups. For most cardiovascular indices, the Ranch Hands appeared to have slightly lower frequency of detectable differences from normal, except for heart murmur and some questionable pulse responses.

More recent publications on the Ranch Hand studies have discussed various endocrine and reproductive evaluations, as related to estimated

TCDD exposure and tissue residues. Because those residues reflect historical 2,4-D exposure as well, negative findings are suggestive of absence of long term effects of all components of the formulation. Henriksen et al (1996) found no evidence of effects on male reproductive parameters in examinations in 1982, 1987 or 1992. Some evidence of increased rate of preterm birth and infant death in offspring of Ranch Hands with the highest exposure was seen by Michalek et al (1998) but the control group also exhibited a high rate. A survey of immune parameters did not indicate a "consistent relation between dioxin exposure category and immune system alteration" (Michalek et al, 1999). A positive relationship between dioxin exposure of Ranch Hands and incidence of diabetes mellitus and glucose metabolism has been found (Henriksen et al,1997), but there is no evidence to suggest involvement of 2,4-D.

Garry et al (1999) measured circulating levels of several hormones before, during and after application seasons for a variety of pesticides. In herbicide applicators there was a slight increase in free testosterone over the course of the season but a decrease compared with the control group. Follicle stimulating hormone was slightly elevated after the season, but herbicide applicators had much lower preseason levels than those who applied insecticides or fumigants. None of these changes appeared to be physiologically significant, and no association with individual formulations was possible. The same group (Garry et al, 2001) have looked for correlation between cellular responses and urinary 2,4-D levels in forestry applicators. The highest 2,4-D levels were found in backpack applicators, who also had elevated levels of luteinizing hormone, but not follicle stimulating hormone or testosterone.

Neurological effects of 2,4-D

Evaluation of potential 2,4-D related neurological effects has not been a specific part of the regulatory toxicology requirement, but other toxicology data indicates that neurological impact is not likely. However, there have been clinical reports of peripheral neuropathy (pain and muscular deficits) in some of the few humans who are known to have been heavily exposed to 2,4-D in suicide attempts and accidents with concentrated material. As a consequence there has been considerable independent research seeking evidence of neural effects, or investigating possible mechanisms by which such effects might occur. In addition, there has been a popular notion that 2,4-D is a potent neurological intoxicant, which also makes it important to discuss the question thoroughly.

While the signs and symptoms in the clinical human cases did not fit well with the established diagnostic criteria for chemical neurotoxicity, there has been sufficient evidence to justify concern about possible long-term neural injury in the limbs. A complication has been that many of the cases included other factors that either contributed to or may have induced the observed effects. Some suicides and attempted suicides were with formulation or mixes containing solvents, in one case about a pint of kerosene. Some cases accompanied pre-existing disease.

There are a few individuals who have been given very heavy doses of 2,4-D under close clinical observation. In 1959 a French physician reported the outcome of a trial use of 2,4-D in the treatment of cancer. 2,4-D was injected intramuscularly at doses up to 2.5 grams daily, or 40 to 50 mg/kg/day, over periods as long as 140 days. At the higher doses, patients were dizzy, depressed, and confused, but there were no persistent symptoms, and no evidence of neural damage (Apffel, 1959). Seabury (1963) treated two patients with advanced coccidiodomycosis with even higher doses of 2,4-D, resulting in muscle twitching and loss of consciousness, but recovery from the effects of the administered chemical was complete in 48 hours. The treatment of the disease in both trials was not successful.

Nerve conduction velocity in workers in factories manufacturing 2,4-D and 2,4,5-T has

been reported to be slower than in a control population. (Singer et al, 1982) The role of 2,4-D in this study is difficult to evaluate because the controls were in a different kind of occupation, and were examined at a different time and location. (Measurement of the speed of a nerve impulse over a long nerve trunk can be measured between electrodes located near the origin of the stimulus and the far end of the nerve.)

The search for evidence of neural toxicity, using animal models, has been based on three general questions:

a. Where does 2,4-D go and what happens to it in the central nervous system?
b. What biochemical and physiological changes in the brain or other parts of the nervous system, if any, are caused by 2,4-D?
c. What functional neurological changes might be induced by 2,4-D?

Central nervous system

Probably the first report of animal research into central nervous effects of 2,4-D was by Desi et al (1962). The work may have been prompted by a report by Goldstein et al (1959) of three patients who had apparently been heavily exposed and who experienced sensory and motor symptoms over an extended period. Desi et al (1962) administered 2,4-D at a dose of 200 mg/kg to rats and detected changes in the electroencephalographic pattern. The neurological effect disappeared in a little over an hour, even though the dose used was high enough to produce observable toxicity.

Elo and Ylitalo (1977,1979) published two reports in which they suggested that 2,4-D damages the so-called blood-brain barrier, which limits the passage of many molecules from the blood to the central nervous system. Close inspection of the paper suggests that the apparent effect resulted from accumulation of 2,4-D in the brain because of the limit in ability to excrete such material from the brain. Doses of 2,4-D (300, 600 mg/kg) that cause severe general toxicity caused damage to capillary blood vessels in the brain of the rat. No injury was detectable at a dose of 150 mg/kg. In the mouse, chicken, rabbit, hamster and guinea pig no vascular injury was evident even at doses that caused severe general toxicity (Elo et al, 1988). Kim et al (1988) have also shown clearly that the blood-brain barrier is not affected at doses that can be tolerated by the subjects.

It is likely that 2,4-D moves into the brain fairly easily from the blood stream. The various esters and amine salts hydrolyse to 2,4-D acid under body conditions, and the acid is the predominant form in the blood. (The term acid should not be interpreted as a highly reactive substance, as we are accustomed to think of inorganic acids such as nitric or sulphuric acids. The term simply identifies certain features of the molecule and is standard chemical terminology.) In this case, the acid is not significantly ionized in the blood, and therefore has little or no electrical charge to impede its movement across membranes. Both fat and water solubility of 2,4-D are limited, but are still sufficient to allow movement through cell walls.

A possible mechanism of central nervous system toxicity relates to excretion from the brain to the general circulation. The mechanism by which 2,4-D is excreted from the brain is almost identical to that in the kidney, already discussed. This "pumping" activity is carried out by a diffuse structure called the choroid plexus. Its normal function is excretion of the organic acids that are metabolic products of neurotransmitter substances. These substances are "pumped" into the cerebrospinal fluid, from which they eventually are exchanged to the blood. This mechanism of excretion from the brain has been known for many years, and has been defined with respect to 2,4-D in a number of reports, perhaps beginning with Pritchard (1980), and including Kim and O'Tuama (1981) and Kim et al (1983,1988). The biochemistry and kinetics of the system have recently been further defined by Pritchard et al (1999).

The system is relatively non-specific and has a finite capacity. If the sum of all of the organic acids it is required to remove is greater than its capacity, excretion of all of the materials will be slowed. This means that 2,4-D in sufficient quantities would interfere with the excretion of the naturally occurring brain metabolic products, and would also impair its own excretion.

This relationship was demonstrated directly by Pritchard (1980) and more recently by Duffard et al (1990). In rats treated with 2,4-D at a rate of about 69 mg/kg/day for 15 or 45 days, brain concentrations of 5-hydroxytryptamine (5-HT; serotonin) and its product 5-hydroxyindolacetic acid (5-HIAA) were elevated, because the total of 5-HIAA and 2,4-D was greater than the system could remove. 5-HT increased because higher levels of 5-HIAA slowed the reaction: 5-HT ---> 5-HIAA. Levels of 5-HIAA and 5HT returned to normal ranges after the treatment was terminated. Levels of these neurotransmitters in offspring of treated rats also were higher. If the pups were foster-nursed on untreated dams, recovery occurred, but if maintained with treated dams through weaning the effect persisted. Unfortunately only one dose rate was employed so it is not possible to judge the threshold above which excretion would be impaired. Elo and MacDonald (1989) did a similar experiment with single doses of 10, 30, 100 and 200 mg 2,4-D/kg and found a dose dependent response, with modest interference with excretion at the highest dose. Among six different neurotransmitter compounds or their metabolites there was a significant effect only on 5-HIAA at 100 and 30 mg/kg. There was no effect at 10 mg/kg.

The competition for removal from the brain has also been demonstrated by experiments in which 2,4-D excretion was inhibited by administration of probenicid, another substance that must be removed by the organic acid "pumping" system. (Ylitalo et al. 1990).

Neuromuscular effects

Neuromuscular effects of a chemical or disease are observed through study of the biophysics, biochemistry and physiology of the nerve and associated muscle, observation of functional activity of the intact animal or through microscopic pathological study of the nerve, muscle and their junction.

One of the earliest published reports on 2,4-D (Bucher, 1946) described a myotonia in mice given single doses of 200 mg/kg, with the animals awake and alert, but demonstrating very poor coordination when moving. (Myotonia is muscular irritability and contraction (spasms) with inability to relax.) They recovered if exercised, but if left quiescent the myotonia persisted. Since that time several papers have been published reporting efforts to define the mechanism of the effect. 2,4-D treated animals were also used as a model for study of congenital myotonia in humans (Ezaguirre et al, 1948; Iyer et al, 1977; 1981). Dux et al (1977) reported altered calcium distribution in muscle; there has apparently been no effort to examine this phenomenon with more modern methods for studying calcium channels and related membrane transport activities. The changes in chloride and potassium conductance across muscle cell membranes in classical myotonia has also apparently not been investigated. The neurological basis for the muscular effect was established by Iyer et al (1977; 1981) by showing that in animals in which nerve connection to muscles was blocked, the myotonia did not occur.

Neurological function in intact animals was studied by Squibb et al (1983) in rats given single doses of 20, 40, and 80 mg 2,4-D/kg twice weekly for five weeks, followed by a six week observation period. Grip strength in both fore and hind limbs increased to some extent, relative to dose and time of treatment, which may relate to the increased contractility associated with the myotonia of larger doses discussed above. Recovery time was also dose related. Other neurological tests were not different from control values.

Toyoshima et al (1985) measured reflexes and nerve conduction velocity in rats on two treatment schedules. One group received 100 mg 2,4-D/kg six days a week for three weeks; the other group received 80 mg/kg three times a week for 12 weeks. The latter dose rate approached lethal levels over the experimental period. There were no significant differences in any of the measured indices, nor was there evidence of faulty reflexes or motor or sensory activity.

In dogs, Steiss et al (1987) made clinical neurology, electromyelography and motor nerve conduction velocity measurements for 28 days after single oral doses of 25, 50, 75, 100 or 125 mg 2,4-D/kg. They found a transitory myotonia after doses of 50 mg/kg or more, and at the higher doses some effects persisted up to three days. A comparison of clinically visible manifestations of myotonia in the dog, and electromyographic (EMG) measurements was made by Beasley et al (1991), who found that single dogs given 175 or 220 mg 2,4-D/kg manifested clinical symptoms as well as EMG evidence. At doses of 8.8 mg/kg, there were EMG differences with no clinical effects. The next lower dose of 1.3 mg/kg produced no detectable effect.

Schulze (1987, 1988) and Schulze and Dougherty (1988a, 1988b) investigated the myoneural effects of the n-butyl and mixed butyl esters of 2,4-D in rats. At doses of 150 mg/kg and above the n-butyl ester caused a greater depression of basic motor control than did the mixed esters. Doses below 100 mg/kg appeared to have no effect on neurological activity. Food deprivation seemed to decrease responsiveness to the chemicals. (Schulze, 1987) Because hydrolysis of the butyl ester to 2,4-D acid and n-butanol occurred rapidly in both cases, it was suggested that the difference in response was due to formation of n-butanol rather than 2,4-D (Schulze, 1988).

Prolonged exposure produced somewhat similar results (Schulze and Dougherty, 1988b), except that two additional findings emerged. Doses of 150–250 mg/kg/day over four 14-day treatment periods separated by 5–8 day rest periods showed that the effects were reversible within 24-48 hours, and the rats became tolerant of treatment by the 10th day.

Rats and guinea pigs given daily doses of 200 mg 2,4-D/kg, divided into three injections for five days, were visibly myotonic. Myotonia disappeared overnight after the end of treatment but there were numerous microscopic changes associated with both degeneration of muscle and attempted repair (Danon et al, 1978)

Exploring the neural basis for the myotonia, Bernard et al (1985) measured acetylcholine esterase (AChE) activity at the motor endplate in rat muscle, where the nerve endings join muscle fibres, following a dose of 200 mg 2,4-D/kg. Acetylcholine is the neurotransmitter chemical released at the nerve ending that carries the impulse for contraction to the muscle cell. AChE removes acetylcholine after each impulse to prevent uncontrolled muscle contraction. (AChE is specifically inhibited by organophosphate insecticides such as parathion.) AChE levels and spontaneous motor activity in rats were decreased after single doses of 200 mg/kg, and returned to normal levels by 48 hours. No effects were seen at a dose of 100 mg/kg.

Virtually all worker exposure to 2,4-D will be on the skin unless it is ingested due to careless eating and smoking hygiene. Mattsson et al (1986a; 1986b) applied a 12% solution of 2,4-D amine to the shaved lower limbs of rats for 2 hours a day, five days weekly for three weeks. (The concentration of a typical spray solution will be 0.5 to 2%.) The applied dose each day was stated to be about 111 mg/kg, which resulted in plasma concentrations three hours after the last treatment of just under 70 ug/ml (70 parts per million). A variety of nerve function tests and electrophysiological measurements were made, with a full pathological evaluation of the nervous system. While the treatment caused skin lesions, decreased body weight and increased kidney

weight, as expected, no neurological effects were observed.

The experiments of Schulze and Dougherty (1988a,b) and Mattsson et al (1986a,b) may be compared on the basis of blood levels of 2,4-D that were reached during treatment. Maximal concentrations on the order of 600 ug/ml (600 ppm) by the tenth day were seen by Schulze and Dougherty, compared with about 70 ug/ml in the Mattsson et al studies. In the latter case, the levels found were approaching a maximum that might be accomplished under dermal administration; in the former the material was fed, a route that can move much larger amounts of chemical into the bloodstream.

It is quite evident that massive intoxication, of an order that cannot be approached in an occupational exposure context, is necessary to produce neuromuscular effects. However, the observations just discussed are useful in considering incidents such as spills leading to heavy exposures to concentrated material without proper cleanup procedure.

A research collaboration in Argentina has studied a number of aspects of potential neural effects of 2,4-D. Much of their work has been oriented toward identifying effects during foetal and postnatal development at relatively high dose rates. Unfortunately, the studies usually do not include dose response data that would permit assessment of effects at occupationally relevant exposures.

Effects of 2,4-D on the immune response

Effects of a non-protein chemical on the immune system can take two general forms. A foreign substance may combine with a protein in the body and the complex may be "seen" by the immune system as a foreign protein. Formaldehyde is an example of such a chemical. The immune system has a form of memory, which prepares the body to produce antibodies if the same complex is encountered again. Such an event is typically called an allergic response, and if severe enough it can be distressing or even life threatening, like an allergy to bee stings. Few non-protein chemicals have such capability. The other general response is damage to components of the immune system such that the organism cannot respond properly to infection or to the kinds of genetically altered cells that may result in cancer.

Present registration protocols do not include direct evaluation of effects on the immune system, but there have been some studies directed at the question of 2,4-D immunotoxicity, and observations of exposed humans. There are also indirect indices that can provide evidence of the absence of immune effects.

Cushman and Street (1982) did a two step study, first constructing a protein conjugate of 2,4-D, then using it to immunize mice, to learn whether it is possible for the animals to respond to the foreign protein. After determining that mice could indeed respond, they set up a skin hypersensitivity assay with 2,4-D in the mice that had developed immune memory. Application of 2,4-D alone to the ear of the mice (approximate dose applied 12 mg/kg) produced no response, and did not result in antibody production. While these findings do not rule out the possibility that occasional humans can develop allergic sensitization, it indicates that the process may be biologically difficult and that other factors must be eliminated if apparent cases are seen.

The ability of 2,4-D to damage the immune system was tested by Blakley and Schiefer (1986). They applied 2,4-D n-butyl ester to the skin of mice at acute doses up to 500 mg/kg, and also applied nine 300 mg/kg doses over a three-week period. Ability to respond to sheep red blood cell antigen (a standard assay) was impaired at the highest doses in the acute tests, but there was little effect from the longer-term treatment. The latter treatment did enhance the response to other stimulants of immune response. All of the immunodepressant effects occurred only in company with severe general intoxication, and were considered by the authors

to be secondary to those changes. The same laboratory (Blakley and Blakley, 1986) investigated the effect on offspring after treatment of pregnant mice with 2,4-D n-butyl ester on day 11 of gestation. (In mice the greatest sensitivity to immune damage is through days 10-12 of gestation.) Doses were as high as 200 mg/kg, but the authors concluded that effects on offspring were so slight that they were "unlikely to be of any immunotoxicological or immunoteratological significance."

Assessment of cellular immune system effects in the heavily exposed Ranch Hands showed no differences between the subjects and their comparison group (Wolfe et al, 1990). The findings of other reviews and literature searches, as summarized by Munro et al (1992) indicate no association of 2,4-D with adverse immune effects.

Perhaps the best practical experimental evidence for the absence of immune effects of 2,4-D arises indirectly from cancer studies and the three-generation reproduction study mentioned later. In any long term, high dose study in which there is no increase in the incidence of infectious disease among the treated animals, it is quite unlikely that significant immune effects have occurred. Except for germ-free colonies, even the most well-managed facilities have a background presence of infectious disease, particularly of the respiratory tract, and a failure of the immune system would allow infection to increase sharply. There appears to be no evidence of such increase in the long-term studies.

It must be concluded that there is no evidence to suggest direct effects of 2,4-D on immune function. As with any chemical, general toxicity produced by very high doses may be accompanied by changes in some measurements of immune function.

Miscellaneous effects of 2,4-D

Energy metabolism

Because of its structural relation to chlorophenols, there has been inquiry into the ability of 2,4-D to uncouple oxidative phosphorylation. (Oxidative phosphorylation is the step in which oxygen is consumed in conversion of foodstuffs into a form of energy supply that can be used in the cell. When uncoupled, oxygen consumption continues, but energy conversion stops.) 2,4-D is not effective except at concentrations that are very high relative to toxic doses (Zychlinski and Zolnierowicz, 1990). The effect of 2,4-D on other steps in the energy conversion process has also been found of little significance (Cascorbi and Foret, 1991)

Effects on enzymes that detoxify foreign chemicals

While 2,4-D does not require detoxication in the body, there has been a question whether it would alter the activity of enzymes that convert other foreign chemicals and molecules such as steroid hormones produced in the body to forms that are more easily excreted. The reason why such activity should be of concern is that detoxication of some chemicals produces reactive intermediate compounds that may contribute to mutation and cancer causation. Increased enzyme activity is a physiological response that has evolved to deal with foreign chemicals in the diet and which also alters synthetic substances for excretion. Changes in activity of some detoxifying enzymes has been observed (Chaturvedi et al, 1991; Dierickx, 1988), but they have no significance at dose levels possible in the workplace.

Effects on Reproduction and Development

Chemicals may affect reproduction and or development through impacts on either parent before conception, during gestation, or even during the immediate postnatal period. The insult may be genetic and passed on to the offspring, or there may be direct toxicity that compromises the ability of the parent to support the developing foetus. Direct effects on the offspring may result in toxicity at certain stages of gestation that result in structural defects or inadequate progress in the timetable of development. Another possibility is alteration of activity of enzymes that remove steroid hormones, with attendant reproductive effects (see preceding paragraph). All of these possibilities can be evaluated experimentally.

2,4-D is not a potent reproductive toxicant. The most frequently observed effects at high doses in teratogenicity studies (evaluation of birth defects) were decreased foetal weight, delayed hardening of bone, wavy ribs and fluid accumulation under the skin. These are not birth defects as such, but are the result of general intoxication of the foetus and the mother. Such changes may occur without decreasing survival or postnatal development. True birth defects are not seen in most species, but there is some evidence of cleft palate and eye deformities in mice. No effects occur in rodents at dose rates below 20 mg/kg/day. In such studies the test chemical is administered daily through days 6–15 of gestation, which is the period during which organs and other structures become differentiated and take form in these species. The corresponding period in humans is from the end of the second week to the end of the third month of pregnancy.

In one three generation reproduction assay in rats reported to USEPA by the registrant, body weights of both pups and mothers were decreased at a dose rate of 20 mg 2,4-D/kg/day. There was no effect at a daily dose of 5 mg/kg, and there was no effect on fertility or survival of pups at the 20 mg/kg/day rate. An earlier similar study reported by Hansen et al (1971) found increased mortality of offspring and decrease in maternal and foetal weight at a daily dose of 75 mg/kg, but no adverse effects at doses of 25 or 5 mg/kg/day. (The multi-generation reproduction assay has been described in the section on toxicity testing. Briefly, parent animals are treated continuously beginning several weeks prior to mating, through gestation, and until the pups are weaned. The pups are then placed on the same treatment from weaning through their reproductive cycle and weaning of their offspring.)

Blakley et al (1989a) exposed female mice to a formulation of 2,4-D and picloram for 60 days prior to mating, then continued the treatment of half of the mice throughout gestation. Dose rates were quite high, estimated at 84, 168 and 336 mg/kg/day of 2,4-D. The concentration of picloram in the formulation is much lower than that of 2,4-D, and the reproductive toxicity of picloram is low, so the study is useful in assessing 2,4-D toxicity. There was evidence of dose-related maternal and foetal toxicity, which is not surprising at such a high dose rate. The incidence of abnormal foetuses increased in the animals treated both before and during gestation. The incidence of abnormals dropped sharply in the low and high dose groups treated only prior to mating, and was approximately at control levels in the mid-dose group. There were no treatments during the gestation period alone. The absence of a dose response indicates that exposure prior to conception does not place offspring at risk from injury to reproductive endocrine systems or structures, nor does it induce genetic damage that would carry forward in the form of foetal anatomical defects.

A companion report (Blakley et al, 1989b) described a study in which males were maintained on 2,4-D for 60 days prior to mating, and bred to untreated females. There were serious discrepancies in the stated dose rates, but as nearly as could be determined, malformations were apparently not dose-related. For example, there was a significant increase in malformations

at the middle dose rate, but at the highest rate there was no difference. All females mated to the males that survived the highest dose rate had 100% fertility, but only 69% of females mated to mid-dose males conceived. Taken at face value the paper provided a weak suggestion of a paternal effect at very high dose rates, but even that response is highly questionable because of the erratic dose-response.

Studies of effects of chemicals on foetal development have usually been anatomical, seeking evidence of change in structure. Biochemical effects on foetal development can also be caused by intoxication with some chemicals, and this possibility has been probed to some extent for 2,4-D. Mohammed and St. Omer (1985) measured monoamine levels in developing rat brain after the dams had been treated with 0, 50 and 100 mg of a 1/1 mixture of 2,4-D and 2,4,5-T/kg/day through days 6–15 of gestation. The monoamines are a class of compounds in the brain that serve as transmitters of impulses between nerve cells. Presumably, changes in their production or removal might alter brain activity in the newborn. Dopamine levels were depressed somewhat at the highest dose. Unfortunately the table and text in this paper are inconsistent; decreases are reported, but evident increases are not discussed. The authors do not see in their work a mechanistic explanation for behavioural changes they have reported in newborn rats following similar treatment.

The same authors (St. Omer and Mohammad, 1987) found a non-dose related decrease in swimming performance, with a dose-related increase of whole brain norepinephrine, and an increase in dopamine at the highest dose. The significance of the finding is unclear because almost a week elapsed between the end of treatment and measurements of possible effects.

Some epidemiological investigation of potential effects of 2,4-D on reproductive outcomes has been made. The most useful was a case-control study of the possible relation of paternal occupational 2,4-D exposure to spontaneous abortion in humans (Carmelli et al, 1981). The report was prepared for the National Forest Products Association and the US Forest Service and is generally recognized as well conducted work. The executive summary of the report captured the findings effectively: "The data on 134 miscarriages and 311 live births did not indicate a positive association between phenoxy herbicide exposure in males and subsequent spontaneous abortions in their wives. Stratifying the data for farm workers and forest/commercial workers also did not show an association. Although the overall comparisons did not support an association between paternal exposures and reproductive problems, in an isolated subgroup of young forest/commercial workers (21 cases and 54 controls) there was a suggestive association with overall 2,4-D exposure, statistically significant at a low confidence level. No association was observed for the same age group in farmers." The plausibility of a true association was diminished by the finding of a difference in the influence of age in the two groups. The authors felt that the finding only indicated that there should be further study.

A comparison of aircraft applicators with their siblings found a higher incidence of problems among the siblings (Roan, 1980). None of the pesticides used was identified, but it may be assumed that they included 2,4-D because of its very wide use. Assessment of reproductive outcomes of Vietnam veterans, in which almost 100 different birth anomalies were considered, (Erickson et al, 1984) led to the conclusion that no effect could be seen.

There is considerable public concern about miscarriage possibly resulting from exposure to herbicides. Miscarriage is a very common problem in the normal human population, with about 15% of known pregnancies ending in spontaneous abortion. The assays of reproductive effects discussed earlier also encompass the toxic impacts that might lead to miscarriage, and indicate no such tendency except at massive doses that result in severe general toxicity to the pregnant females.

2,4-D is not recognized as a significant teratogen because few studies have found effects at doses other than those causing maternal toxicity.

Mutagenicity, Genetic Toxicity and Related Cellular Effects of 2,4-D

The various adverse effects of 2,4-D discussed above are all threshold based, meaning that there is a dose rate or level below which there will be no response. Genetic effects may or may not have a threshold, although they are certainly dose responsive. As the dose rate decreases, so does the probability of an effect, and as a practical matter, at very low doses the probability becomes so low that it is virtually equal to zero.

There have been many studies of the ability of 2,4-D to cause mutations, or genetic toxicity. Mutations are changes in genetic material (DNA) that can be transmitted to a later generation of the whole organism if germ cells are affected. In other tissues the change may be transmitted to new cells of the same tissue when the altered cell divides. Many tests for mutagenic ability are conducted with bacteria, yeast, mammalian cells in culture or with insects, and they can be completed in a short time. The objective is to learn whether the chemical has the capability to alter DNA under purposely maximizing conditions. Such tests do not indicate the specific probability that a chemical might cause mutations in mammals.

Almost all of the mutagenicity assays have been negative, and the few tests that were not clearly negative indicated a marginal response, and in most cases other similar assays showed no effect. The nature of genetic toxicity assays is such that apparent positive or negative results may occur in any series, for reasons unrelated to the activity of the chemical on genetic material in the cells. The remarkably large number of mutagenicity assays on 2,4-D prior to 1984 are tabulated in Newton and Dost (1984).

In spite of the extensive catalogue of mutagenicity assays of 2,4-D, there are still regulatory and research attention to the subject. Many of the earlier tests, while informative, do not conform to present regulatory guidelines, which are designed to both elicit information and put it in a format that enables comparison of effects among chemicals. The subject is still of research interest because of the wide use of 2,4-D, and possibly because some investigators believe the very limited suggestions of genetic activity may be strengthened if a sufficiently sophisticated test system can be devised. Much of the more recent work is with tests that are not yet recognized in regulatory protocols, or are modifications of standard systems.

Schop et al (1990) have compared the well established bone marrow micronucleus assay for disorganization of chromosomes with a newer method called the nuclear aberration assay which detects a greater range of disturbances of chromosomal structure. It also may be applied to other tissues than bone marrow. In this case hair follicles in the skin were the test structures, which may be more sensitive to dermal exposure. The lowest effective dose was about 440 mg 2,4-D/kg, applied to the skin for 24 hours. If one assumes 5% absorption the systemic dose would be about 22 mg/kg. The prospect of such a dose occurring in a worker is remote in the extreme. Exposures of workers and the doses that result from exposure are discussed in detail in another section. Even careless applicators are not likely to be in skin contact with more than 5 mg/kg, of which only a small fraction will be absorbed.

In contrast to the dose response findings by Schop et al (1990), Turkula and Jalal (1987) reported that rats are exquisitely sensitive in a similar assay. They claimed to find significant effects at doses of 0.075 mg/kg or higher, 24 hours after exposure. The meaning of these conflicting results is not clear. Of several reports on tests of chromosomal integrity after 2,4-D treatment *in vitro* and *in vivo*, the only one that even approaches this level of sensitivity was reported in the Soviet literature (Pilinskaya,

1984), with insufficient detail to judge just what was done. Other work shows minimal or no effects on chromosomes. (Korte and Jalal, 1982; Bongso and Basrur, 1973; Magnusson et al, 1977; Jenssen and Renberg, 1976). The unusual effects reported in Turkula and Jalal (1987) could be typographical error, since there are a number of proofing errors and several inaccurately quoted references in the publication.

Phenoxy herbicides mimic the action of certain plant growth-regulating auxins, which are able to interfere with recombination of chromosomes in fungi used as test species for genetic toxicity. Kappas (1988) reported comparisons of these substances with a group of phenoxy acids in standard bacterial mutagenicity assays and a mitotic recombination assay in the fungus A. nidulans. In one of the six strains of Salmonella, 2,4-D was weakly mutagenic, but only after incorporation of liver enzymes that metabolize foreign chemicals into the assay. This is a curious finding, since 2,4-D is not metabolized in mammals. A slight effect after activation was also observed in the assay for mitotic separation at relatively low concentrations of 0.9 - 10.6 mg/litre. A similar response to MCPA, another phenoxy herbicide, occurred only at concentrations 60 fold higher.

Rivarola and Balegno (1991a, 1991b) observed an effect of 2,4-D on synthesis of DNA and protein in cultured Chinese Hamster ovary cells, a common experimental preparation. Cell cultures have the advantage (and disadvantage) of being free of all of the modulating influences of regulation by an intact organism. It is therefore possible to maintain very high concentrations of test chemicals over an extended period, to learn qualitatively whether an agent can have an effect on genetic structures. 2,4-D at a concentration of 220 mg/litre was found to inhibit DNA and protein synthesis, as well as cell growth. The effects reversed when 2,4-D was removed from the system. At similar concentrations, Zhao et al (1987) found no inhibition of DNA synthesis in cultured mouse cells. A concentration of 220 mg 2,4-D/litre is in excess of any level that can be achieved in even a severe dermal exposure of a human or an animal model. For example, data from Pelletier et al (1989) indicates that a dermal exposure of rats to 10 mg 2,4-D amine/kg would result in a maximal level in body fluids of roughly 0.2 mg 2,4-D/litre of body fluids, which would be sustained over several hours. There have been no findings in intact animals that suggest an effect on synthesis of DNA or protein.

In a system designed to show direct damage to DNA, as distinguished from potentially transmissible genetic effects, Clausen et al (1990) used cultured human fibroblasts to compare cellular toxicity of technical 2,4-D acid, 2,4-D dimethylamine (DMA), 2,4-D trimethylamine (TMA), and a commercial formulation of 2,4-D-DMA. The lowest concentration reported was 5 millimolar, which is 1100 mg per litre, and which had little effect. 2,4-D acid did not interfere at concentrations of 4000 mg/litre, and that is the form to which all salts and esters hydrolyse once in the blood stream. In a purified solution of DNA, 2,4-D and its DMA salt at concentrations of about 2200 mg/litre caused breaks in DNA, but the TMA salt was ineffective. An examination of the effects of realistic concentrations would have been useful.

The weight of the evidence indicates that the ability of 2,4-D to cause mutation or genetic defect is very limited. At maximum doses that can be encountered in the workplace such effects are extremely unlikely.

The absence of appreciable mutagenic activity is important for two reasons. There has always been a significant background of genetic defect in the human population. It is essential to assure that human activities do not add to that burden. This kind of information is also important in evaluating the ability of a chemical to cause cancer. The mechanism by which direct acting carcinogens (those that cause change in cellular DNA) cause cancer involves a sequence of mutations, that eventually result in clones of cells in which control of growth, division and

differentiation have been lost. There is a practical although by no means perfect correlation between ability to cause mutations and ability to cause cancer. A chemical that is not mutagenic is not likely to cause cancer by this mechanism.

Carcinogenic Potential of 2,4-D

The early animal assays of the carcinogenicity of 2,4-D were negative, but they were inadequate for a variety of reasons. New carcinogenicity investigations in mice and rats have been completed for the purpose of reregistration of 2,4-D by USEPA. (Reregistration is a process by which registered products are re-evaluated for compliance with current regulatory standards.)

Several epidemiological investigations of human populations presumably exposed to phenoxy herbicides were conducted in the seventies and early eighties. There have been serious criticisms of methodology and other deficiencies in many of these studies, but they had the great value of stimulating many other stronger studies that are still appearing in the literature. The two groups of cancers that appeared to change in frequency were soft tissue sarcomas and lymphomas, but the studies of the period were not consistently positive or negative, leaving the question a matter of argument without resolution.

The cancer assays in the laboratory can be described in straightforward terms, but the epidemiological data is massive and still inconclusive. Several investigations that relate to biochemical mechanisms by which 2,4-D might conceivably contribute to carcinogenic activity have also been carried out.

Evaluation of experimental and epidemiological findings by expert panels

Several expert groups were convened in the late eighties and early nineties to examine the accumulated evidence and develop a weight-of-evidence judgement of the carcinogenicity of 2,4-D. Most considered both experimental and epidemiological evidence.

At the request of the Ontario Pesticides Advisory Committee, the Ontario Minister of the Environment appointed a five-member expert committee to evaluate, through the offices of the Canadian Centre for Toxicology at the University of Guelph, the existing evidence on carcinogenicity of 2,4-D. (Canadian Centre for Toxicology, 1987) In 1989, the Harvard School of Public Health assembled a workshop of thirteen toxicologists and epidemiologists (including the author of the present paper) to consider "The Weight of the Evidence on the Carcinogenicity of 2,4-D" (Ibrahim et al, 1991). Funding was provided by the National Association of Wheat Growers Foundation. At about the same time, a group of independent consultants (Munro et al, 1992) were commissioned by the several manufacturers of 2,4-D to independently review the evidence relating to the safety of 2,4-D. In April of 1993 the United States Environmental Protection Agency Science Advisory Board convened a special advisory panel to consider the question once again, with the objective of determining whether the registration of 2,4-D should be put into special review.

The respective conclusions of these expert groups are discussed later. The extensive scientific expertise represented by these reviews makes it pointless to construct an additional full review for the purpose of this report, especially since the conclusions of the various groups are similar. Several other reviews and commentaries are also discussed. Along with those conclusions, a number of newer epidemiology studies are tabulated to show something of the nature of such research.

Carcinogenicity bioassays

Animal studies conducted in the mid-eighties according to protocols required in the re-registration process, showed no carcinogenic response in mice, but in male rats only there was a low incidence of a tumour in the brain called an astrocytoma. The various review panels considered the effect to be unrelated to treatment. The question of possible causation of astrocytomas has been resolved with new studies commissioned by the registrants and submitted to regulatory authorities in Canada and the U.S. Because there were suggestions that the maximum tolerated dose (MTD) was not reached in the earlier studies, the most recent work employed doses three times higher than the previous assays. There is no evidence of astrocytomas, or other carcinogenicity. (L. Hammond, personal communication, 1995)

The comments of the Canadian Centre for Toxicology (1987) panel on the early finding of astrocytomas in male rats are instructive. They conclude that "while it is not possible to discount this evidence for carcinogenesis," "there is insufficient evidence to be certain that the brain tumours found were related to 2,4-D exposure." The reasoning behind this conclusion lies in the basic biology of brain tumours, and in the behaviour of 2,4-D in the body.

The biological reasons were:

- There was no evidence that the tumours in treated animals developed more rapidly than in untreated animals.
- The increase was limited to only males receiving the highest dose.
- There was no evidence of early pre-cancerous changes in the brain that are expected from chemical carcinogens.
- The tumours were solitary; that is, there was no clustering of tumours in any animal, as would be expected as a result of treatment with a chemical carcinogen.
- The tumours in treated animals were not more advanced than those in untreated animals.

The fact that 2,4-D has insignificant ability to interact with genetic material, and the fact that it is excreted rapidly without forming reactive products in the body also indicate absence of any direct carcinogenic activity.

The Harvard panel was also not convinced that the animal data discussed above demonstrated a cause-effect relationship between tumours and 2,4-D treatment. Because the panel did not believe the study in mice employed a maximum tolerated dose, it was felt that new experiments should be conducted to resolve that question. (Cancer studies are typically conducted with at least two dose levels, the highest of which should be the highest level the animals can tolerate without significant non-cancer toxicity.

The EPA Science Advisory Panel concentrated on epidemiological data but did acknowledge that the finding of astrocytoma in male rats may not represent a treatment related effect. The Panel commented that the higher dose studies then underway would clarify the matter. (Those studies are now complete, and no carcinogenic effect was found, even at three times the dose rate of the earlier work.) (Science Advisory Board, 1994).

Munro et al (1992) concluded that, "the available mechanistic studies provide no plausible basis for a hypothesis of carcinogenicity." By "mechanistic," Munro et al mean all studies that have a relation to possible development of cancer. Metabolic fate, genetic toxicity and mutation, specific organ effects, even reproductive responses all have potential to shed light on the ability of a chemical to cause cancer. The report by Munro et al (1992) is an extraordinarily detailed review and analysis of the toxicology, epidemiology and exposure data. The panel of five peer reviewers of this report are all internationally recognized scientific authorities, and are identified in the publication, a most unusual step, probably prompted by the fact that the review by the Munro group was requested by the manufacturers.

Other experimental findings that provide information about the carcinogenic potential of 2,4-D

The absence of metabolic change and rapid excretion of 2,4-D are discussed elsewhere. They are important to judgement of carcinogenicity, because chemicals that are rapidly moved out of the body are not likely to be carcinogenic; neither are chemicals that undergo no metabolic change prior to excretion.

An area that has elicited some interest is the possibility that 2,4-D may act like a group of other compounds that resemble phenoxy herbicides and cause liver tumours. The suggested mechanism is through increased oxidation of fatty acids in cells, resulting in excessive production of hydrogen peroxide, beyond the normal cellular capacity for inactivation. If not removed quickly enough, hydrogen peroxide forms highly reactive oxygen radicals that may damage DNA. A variety of approaches have been taken to learn whether such a response could support a reasonable hypothesis of potential carcinogenic activity by 2,4-D, but no significant evidence has emerged. (Vainio et al, 1983; Bacher and Gibson, 1988; Mustonen et al, 1989; Kozuka et al, 1991; Mikalsen et al, 1990a, 1990b; Abdellatif et al, 1990).

2,4-D also did not influence the incidence of tumours resulting from spontaneous mouse leukaemia virus infection at doses up to 50 mg/kg/day (Blakely et al, 1992). Average survival of animals at the highest dose was actually about 12% longer than survival of untreated animals. Pulmonary adenomas induced by urethan were increased in 2,4-D treated animals, however.

Epidemiology studies

2,4-D has been the subject of more extensive epidemiological research than any other pesticide, and possibly any other chemical other than the mixture of chemicals in tobacco smoke. About 100 different studies have been done, ranging from those investigating manufacturing workers, to those known to use 2,4-D, to populations of people who happened to be in circumstances where exposure to 2,4-D might have occurred.

Because a major thrust of the several reviews mentioned earlier has been evaluation of epidemiological work, particularly the more recent and pertinent studies, it is useful to provide an overview of such observations that relate to 2,4-D.

Epidemiology is the study of relationships between disease and environmental factors of some kind. Sometimes an apparent occurrence or increase in incidence of a disease condition stimulates a search for factors that might be associated and therefore possibly causal. Alternatively, there may be an attempt to learn if a recognized environmental situation such as water pollution or a hazardous waste site is contributing to an increase in disease.

Almost always, epidemiological investigation is retrospective, a form of after-the-fact detective work, sometimes extending decades into the past. A case of cancer diagnosed in the present may have resulted from exposure that occurred or began 15-40 years earlier; because of such a long latency, much needed information is not recorded precisely, or may be lost, or must be supplied by human memory. This is particularly true of attempts to associate pesticide use decades ago with cancer or other disease states. In past times, disease identification, particularly on death certificates, was often incorrect. Present day diagnoses are reasonably well confirmed, but it still is usually necessary to rely on memory of pesticide uses and exposures, which can be difficult after perhaps thirty years. When the subject is deceased and information must be provided by surviving members of the family the problem worsens. Johnson et al (1993) compared responses of proxy respondents in two studies to those of the cases themselves and found that most of the apparent

increased risk arose from proxies rather than the cases.

A more informative approach is the prospective study, in which a population that might be at risk is selected and followed through a long period to learn if they have a higher incidence of disease than unexposed controls. Because the group is identified at the beginning or soon after the exposure or other impact instead of many years later, it is possible to collect detailed environmental, occupational and medical information through out the study. It is rare that the need for such investigation can be predicted.

Two prospective studies that can provide information on 2,4-D are in progress. The Operation Ranch Hand investigation has been under way since shortly after the Vietnam War, with periodic analyses of findings. The Ranch Hands were the unit responsible for handling and spraying herbicides in Vietnam and have been followed closely since that time. They were exposed to vastly greater amounts of 2,4-D than any other personnel, or people engaged in agriculture or forestry. They were also much less exposed to some of the confounding factors experienced by ground troops. Both the Ranch Hands and their controls, air crew personnel not exposed to the herbicides, are examined in great detail periodically. The Ranch Hands have a slightly greater frequency of basal cell carcinoma (a form of skin cancer) but there are no other significant differences between the groups. (Wolfe et al, 1990; Michalek et al, 1990) There are less than 1000 subjects in the Ranch Hand group, which is too few to assure that there is no increase in rare cancers; fewer than one case of soft tissue sarcoma or non-Hodgkins lymphoma can be expected in either group. There also may not yet be sufficient time elapsed to account for latency. Nonetheless, the overall absence of significant differences between this group and their controls is reassuring.

Even this detailed work has its problems, because the exposures included 2,4,5-T and its contaminant tetrachlorodibenzo-p-dioxin (TCDD). If some positive relation is found there will remain the question of the respective possible role of each component. There are certain unique characteristics of TCDD effects that may enable partial resolution of that question if effects are eventually seen. (Conversely, if the findings continue to be essentially negative, they have implications for judging health impact of all components of Agent Orange.)

The other ongoing prospective study is the Agricultural Health Study (AHS), a joint effort by the National Cancer Institute, the National Institute for Environmental Health Sciences, and USEPA. Health histories and various potential risk factors for agricultural populations in Iowa and North Carolina will be followed for many years or until death, in a group of related studies. Among other factors, usage of more than 100 pesticides will be included. The program has been described by Alavanja et al (1996). A committee convened by the Harvard Center for Risk Analysis has prepared a critique and recommendations for strengthening the study (Gray et al, 2000). It is too early to expect useful information from this very broad effort.

With the uncertainties that characterize epidemiological study, it is not surprising that studies of apparently similar exposure situations fail to provide similar answers. The question of carcinogenicity of the phenoxy herbicides, particularly 2,4-D, is such a case.

In spite of the large number of investigations it may never be possible to make a clear statement that there is, or is not, an increased carcinogenic risk associated with the use of 2,4-D. It is likely that any other substance that did not demonstrate consistently positive outcomes would be burdened with the same uncertainty. The most general problem arises from the philosophical difficulty that a negative outcome is more difficult, perhaps impossible, to verify than a positive finding. It cannot be proven that an event will not occur. A more specific problem is that no study relating to 2,4-D has been able to exclude other confounding factors that could be

responsible for a given finding. By evaluating both epidemiological and experimental data, however, it is likely that a consensus of professional judgement of the risk that may be associated with 2,4-D use can be reached. The conclusions of the expert panels identified above are remarkably consistent with respect to epidemiological data, as will be seen below.

The earlier studies of human populations (published prior to about 1985) that may have been exposed to 2,4-D or phenoxy herbicides in general are not discussed here. Because of various deficiencies, particularly uncertain exposure histories and inconsistent diagnoses, the early work has been found to be of limited use in reaching a conclusion about the ability of 2,4-D to cause cancer in exposed groups of humans. While the subject of much public attention, their primary value has been in focusing attention on the question, and several more recent efforts have at least partially overcome the shortcomings of early investigations. The older work is thoroughly reviewed by Munro et al (1992).

Most of the attention in the investigations of 2,4-D has focused on two broad classes of cancer, lymphomas and soft tissue sarcoma. Lymphomas arise in lymph nodes and in lymphoid tissues in other organs. These structures contain and release into the bloodstream cells that are essential to defence against infection and cancer, by creating antibodies and by directly intercepting foreign cells and particles. Study of these diseases is complicated by the many forms of Hodgkin's (HD) and non-Hodgkins lymphomas (NHL), as well as soft tissue sarcomas (STS) which are a group of cancers of connective tissues. Kelly and Guidotti (1991) point out that there are 13 kinds of soft tissue sarcomas, and eight of the classes contain a total of 35 subclasses. There are five classes of malignant lymphomas, including Hodgkins disease, which has 11 subclasses. One of the other four classes has five subclasses. The point of discussing this variety is that each of the classes and possibly the subclasses may be considered a different disease. Chemical carcinogens usually cause a narrow spectrum of cancer types, not an indiscriminate pattern. To attribute several kinds of STS, or STS and several lymphomas, to a single chemical makes questionable biological sense.

A further statement by Kelly and Guidotti (1991) is instructive, particularly relative to lymphomas, because the lymphatic tissues and cells are part of the immune system. "Present systems of categorizing the lymphomas, especially, for epidemiological purposes are probably almost useless in the interpretation of the carcinogenic behaviour of chemical exposures because the immune system is so highly structured that it is likely that such effects are targeted in a very specific way, causing a specific kind of lymphoma to the exclusion of others. We advocate a study in which cell surface antigen markers are used to specifically identify the lymphoma by a consistent typology, or the clinical system used by the National Institutes of Health (Reference 84). We believe that terms such as lymphosarcoma or reticulosarcoma are no longer useful." (Reference (84) in their paper is Robb-Smith, 1982.)

There has been a suggestion that brain tumours may have appeared in animal experiments with 2,4-D (see above), but higher dose experiments have not shown such a response, and there is no evidence of increased brain tumour incidence in observations of human populations.

There are major and unavoidable sources of error in these studies of 2,4-D and possible cancer. As noted above, it is very difficult to determine past exposure to a pesticide. Almost all tumours have latencies of decades between the event(s) leading to initiation and eventual clinical detection. Even if usage is known as fact, or the individual was in an occupation in which herbicides may have been used, there is not likely to be enough information to estimate exposure or dose. There are inherent difficulties in remembering over the intervening years what chemicals were used, how much, how frequently

and the use of protective garments. The problem of reliance on the memory of next-of-kin where individuals have died has been discussed above.

There is a remarkable array of other factors that can compromise an epidemiological study of 2,4-D. Farmers, and to a lesser extent foresters, use other pesticides, fertilizers and fuels, and are exposed to other factors such as infectious agents. Even when known, multiple exposure is quite difficult to deal with. The diluents used to carry pesticides, including herbicides, must also be considered as potential contributors to an apparent effect. Contaminants that existed decades ago, but are not in current products, are untraceable, but may have left an impact. Effects from contaminants such as 2,4-dichlorophenol and the limited toxicity chlorodioxins that have been found in 2,4-D are not likely factors in use of present day products, but early production of the herbicides might imaginably have contained other substances that could explain the suggestive evidence obtained about exposures in the post-war period. Much of the production of 2,4-D in earlier years, when exposures occurred that are now being associated with mortality in some way, was in manufacturing plants that also produced 2,4,5-T, with its contaminant TCDD. An example is the ongoing study of a population of manufacturing workers in the Netherlands most recently reported by Hooiveld et al (1998).

Still another possibility is association of lymphoma with some kind of animal borne virus. There is experimental evidence for viral involvement in certain animal lymphomas, but as yet no real suggestion that such disease in humans is likely.

The epidemiology studies have produced mixed evidence, but the various review groups have been quite consistent in their evaluations.

The conclusion of the Ontario panel on the epidemiology data is summarized:

> "Based on the available epidemiological studies 2,4-D cannot be exonerated as a reason for the excess cancer risk seen in studies involving the phenoxy herbicides conducted in the U.S, Denmark and Sweden, but neither can these studies identify 2,4-D as the causative agent. Overall, the epidemiological evidence indicates that a relationship between an increased risk of soft tissue sarcoma and non-Hodgkins lymphoma with phenoxy herbicide exposure is tenable: However, in regard specifically to 2,4-D, the evidence for human carcinogenicity must be regarded as inadequate." (Canadian Centre for Toxicology, 1987)

The Harvard assessment was made two years after the report of the Canadian group, but even with additional evidence then available, the conclusion was not a great deal different. The 13 members of the panel were asked to review the published epidemiology and categorize 2,4-D as a "known," "probable," "possible" or "unlikely" carcinogen in humans, as noncarcinogenic. Quoting the abstract:

> "The predominant opinion among the panel members was that the weight of the evidence indicates that it is possible that exposure to 2,4-D can cause cancer in humans, although not all of the panelists believed the possibility was equally likely: one thought the possibility was strong, and five thought the possibility was remote, leaning toward unlikely. Two panellists believed it unlikely that 2,4-D can cause cancer in humans." (Ibrahim et al, 1990)

The group considered that the association between 2,4-D exposure and non-Hodgkins lymphoma was suggestive but far from established, and that there is little association between exposure and soft tissue sarcoma or Hodgkins disease, and no evidence of association with any other form of cancer. The panel expressed a conviction that any risk that may be associated with 2,4-D falls only upon those who are handling the material, and that use of proper clothing and handling procedures can minimize the risk.

The term "possible" should not be misread in this context. An epidemiological study cannot demonstrate an absence of effect. For that matter, no experiment of any kind can "prove" that an event cannot happen. Whether among a population or in the laboratory, it can only be shown that an effect was not seen, not that it is impossible. The fact that some studies report an increased risk leaves little option but to acknowledge possibility of effect, regardless of the considerable margin of error inherent in epidemiological methods or a specific study, or the biological implausibility of a carcinogenic response.

The EPA Advisory Panel of 1993 has concluded that while some evidence exists for occurrence of non-Hodgkins lymphoma in circumstances where 2,4-D exposure was likely, there is not sufficient data to indicate a cause-effect relationship. There was further concern that in none of the studies could the estimated risk be isolated from other aspects of agricultural work. (Science Advisory Board, 1994)

Munro et al (1992) conclude that "Taken together, the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer."

The reports of the various review groups were efforts to bring the strength of combined and consultative expert judgement of existing data to bear on the question of potential 2,4-D carcinogenicity.

An international effort to answer the question of an association between chlorophenoxy herbicides (and chlorophenols) and cancer of any kind was undertaken by a team of 15 scientists from 13 institutions or agencies in 11 countries (Saracci et al, 1991). Rather than interpreting individual research reports, the group used an international register of 18,910 production workers and sprayers and developed an independent analysis. Many of the individuals had been subjects in other studies, but by bringing them into a common framework it was considered likely that a greater sensitivity could be attained. They used cause-specific national death rates as reference. In other words, findings for Italian workers were related to Italian background statistics, British to British and so on. Only one group was from Canada, none from the US. They point out that interpretation will improve as the period of observation increases (latency), which is true of any cancer study that is extended over time.

Saracci et al (1991) conclude: "The pattern so far indicates no increased mortality for neoplasms (cancers) in general, for the most common epithelial cancers, and no clearly detectable excess for non-Hodgkins's or for Hodgkin's lymphoma. The significant six-fold excess of soft tissue sarcomas occurring in the period 10-19 years since first exposure in the whole cohort, rising to a nine fold excess in sprayers, is compatible with a causal role for chlorophenoxy herbicides, though not specifically for those probably contaminanted with TCDD."

The same group followed this analysis in 1995 with an updated worker population of about 21,000 individuals, using a somewhat different methodology, and again identified a higher apparent risk of 10.3 for soft tissue sarcoma (10.3 times greater than unexposed workers), but not non-Hodgkins lymphoma (Kogevinas et al, 1995). However, the confidence interval ranges from 1.2 to 91. That is, the number really lies somewhere between 1.2 and 91. Usually a lower limit close to one suggests a poor association, as does the wide range. One of the groups who make up the population discussed by Kogevinas et al is a cohort of workers who manufacture herbicides in The Netherlands. Bueno de Mesquita et al (1993) calculated standard mortality ratios for these workers and concluded that "These findings suggest that the increases in cancer mortality among workers exposed to phenoxy herbicides and chlorophenols may be attributable to chance."

There have also been other attempts by single research groups or consortia to understand the meaning of the multitude of studies that have been done.

E.S. Johnson (1990) brought together the many studies that have been done on cohorts of people exposed occupationally, seeking a possible association of phenoxy herbicides and chlorophenols with soft tissue sarcomas and malignant lymphomas. In introducing the paper, he also assembled an extensive overview of all other information related to the question. His reason for concentrating on cohort rather than case control studies is that he believes they minimize bias in estimation of exposure because exposure is identified before the outcome. Case-control studies identify cases of the disease in question, then try to learn if there are a greater proportion of those cases that are associated with some environmental or occupational factor.

E.S. Johnson (1990) concludes that an unambiguous evaluation is not possible yet for two primary reasons. First, there has not been sufficient time elapsed (latency) for the studies to show an effect if it exists. If one case of a given tumour type is expected in every 100 lifetimes in a typical population, the absence of an excess in 100 people 25 years after exposure may have no meaning. Unfortunately, because tumour incidence is a matter of probability, the occurrence of one tumour in excess may also have no meaning; it could very well be just a chance occurrence.

Second, and possibly more important than insufficient latency, E.S. Johnson points out that none of the studies has adequately accounted for concomitant exposure to other chemicals. He characterizes the problem as intractable because other exposures cannot be known, and states "-- it seems plausible that some of these other exposures, which were not all adequately controlled for in these studies, could well account for at least a significant part, if not all, of the apparent association."

C.C. Johnson et al (1990) conducted meta-analyses of cohort and case control studies through 1987 that reported evaluation of possible associations between phenoxy herbicide (PH) and chlorophenol exposures (CP) with soft tissue sarcoma. Meta-analysis is a statistical technique that presumably permits bringing together many research reports to therefore increase the sample size and decrease uncertainty.

The difficulties of dealing with different sample sizes, diagnostic criteria, statistical methodology, exposure criteria, possibly even different research objectives are daunting. Even with those problems, the approach is one crude way of trying to understand what a large mass of research is telling us. Perhaps a visual inspection of the brief descriptions of the studies in Appendix A constitutes a very simple meta-analysis. A more appropriate term would be "systematic review."

The conclusion reached by C.C. Johnson et al (1990) with respect to both sets of investigations was: "In summary, this assessment does not provide strong evidence that exposure to PH and CP is associated with occurrence of STS." (PH, phenoxy herbicides; CP, chlorophenols) The cohort studies together indicated a proportionate mortality ratio of 3.5 with a 95% confidence interval (CI) of 0.7 to 10.3. The wide range and a low end less that 1.0 do not argue for a profound effect. Case control studies provided an odds ratio of 1.1 (CI 0.9-1.4), which is also too close to unity to suggest a genuine response, although the confidence interval is quite narrow.

Bond et al (1989) undertook a similar task, considering only phenoxy herbicides, and including studies of STS, NHL and HD. They described the methodologies of the various studies, discussing differences in exposure criteria, study methodology and distribution of other risk factors. Their conclusion was consistent with that of most other reviewers: "The total weight of evidence currently available does not support a conclusion that any of the phenoxy herbicides present a carcinogenic hazard to man."

The inability of epidemiological studies to provide definition is evident both in differences in study outcomes and conclusions by reviewers of existing data. For example, the very large study by Saracci et al (1991) reported increased

risk for soft tissue sarcoma. However, the various review panels as well as C. Johnson et al (1990) and Bond et al (1989) argue that there appears to be little evidence for an association of phenoxy herbicide exposure with STS. The review by Kelly and Guidotti (1989) also concluded that evidence for an association with STS was weak, and that the evidence for association was strongest for NHL. Even that connection represented only a weak potency. While perceiving a considerable increased risk for STS, Saracci et al (1991) saw no association with NHL. While most reviewers appeared to believe that an association with NHL is possible but weak, Morrison et al (1992) state that their review of the literature "shows reasonable evidence suggesting that occupational exposure to phenoxy herbicides results in increased risk of developing non-Hodgkins lymphoma."

These papers, and the multitude of others on the subject, lead to inconsistent signals. Carcinogenic chemicals tend to produce a limited range of cancer types. If one looks at data on substances that are known to a reasonable certainty to cause cancer in humans, their patterns are rather consistent. Benzene, tobacco smoke (which is really a broad mixture) asbestos, benzidine dyes, arsenic are all examples of reasonable consistency. It is difficult to see a rationale for a single substance to produce two distinctly different kinds of cancer, in a pattern where sometimes one kind appears in one population and sometimes the other appears in another group. There are virtually no other associations.

Less attention has been paid to cancer types other than STS and the lymphomas. Wiklund et al (1989) analyzed cancer incidence in a cohort of 20,245 applicators of pesticides in agriculture whose licenses were issued between 1965 and 1976. These individuals were not identified according to the kind of pesticide applied, but applicators tend to work with a variety of chemicals, certainly including phenoxy herbicides. Total cancer incidence was less than experienced by the population at large. Most cancer types were lower than the expected frequency, but there were slight increases for Hodgkin's disease, endocrine, skin and nervous system tumours. Wiklund et al (1989) also refer to other of their studies, seeking evidence of STS in one and NHL in another. No statistically significant elevation of risk was found in either case.

The inherent problems of epidemiological study where exposure is poorly understood is shown by the great variety of cancer sites and types that can appear to have some connection with herbicide use. Schreinemachers (2000) possibly unwittingly illustrates the uncertainty in the abstract of a study of cancer mortality in northern wheat producing states. Acreage in wheat is taken as a surrogate for phenoxy herbicide use. "The cancer sites that showed positive trends of increasing cancer mortality with increasing wheat acreage were oesophagus, stomach, rectum, pancreas, larynx, prostate, kidney and urethra, brain, thyroid, bone, and all cancers (men) and oral cavity and tongue, oesophagus, stomach, liver and gall bladder and bile ducts, pancreas, cervix, ovary, bladder, and other urinary organs, and all cancers (women)." This list of sites says nothing of the great number of kinds of cancer that might be found at each site. This is really an index of the vast array of independent and interactive risk factors that can be associated with any given life style. For a given chemical to have such an encompassing impact would require an astonishing potency. Certainly, 2,4-D does not.

Association of pesticide use with primary lung cancer appears questionable. McDuffie et al (1988; 1990) evaluated lung cancer cases among farmers in Saskatchewan; they report "an absence of correlation of lung cancer risk with occupational exposure to any specific substances or pesticides grouped by chemical composition." It was interesting as well that this group found no causative interaction between farming exposures and cigarette smoking. That is, farming factors did not increase the risk of those already more sensitive because of smoking. In this study, smoking was the predominant causal influence of lung cancer. However, Becher et al

(1996) reported an increase in mortality due to respiratory cancer among manufacturing workers exposed to phenoxy herbicides and dioxins. Other possible risk factors were present as well.

Leukaemia appears to have no association with herbicide use (Wiklund et al, 1989) but farmers as a group were found by Brown et al (1990) to have slightly increased risk of leukaemia. More substantial risks were associated with animal insecticide use.

There have been a few efforts to find possible associations between herbicide exposure and cancer in domestic animals. Newell et al (1984) examined more than 20,000 female sheep at slaughter, seeking associations between various environmental factors and the relatively common (in sheep) small intestinal adenocarcinoma. Among the factors found associated with increased incidence were use of herbicides on pasture areas. 2,4-D was not among the phenoxy herbicides that appeared to have an effect. There were substantial regional variations. Also, confusing the findings, is a positive association with an herbicide that is recognized in both Canadian and US regulatory policy as having no evidence of carcinogenicity, and a negative association with an agent that is a suspect carcinogen.

A publication of findings of malignant lymphoma in dogs associated with use of 2,4-D in lawn care Hayes et al (1991) has drawn considerable attention. The study had the advantage of a large sample of animals. The analysis included three groupings: animals exposed to lawn care chemicals by commercial application by owner application, and those in which both commercial and owner application were done. A fourth analysis combined all groups. Only the latter grouping was estimated to have an increased risk, with an odds ratio of 1.3 (95% confidence interval, 1.04-1.67), which is barely significant. There are serious deficiencies in the estimation of exposure; stated frequency of herbicide use by dog owners as determined by interview is at variance with known use patterns, and the wide selection of chemicals that usually accompany 2,4-D in lawn care preparations was not accounted for. There was no attempt to identify which chemicals the commercial applicators used.

A critique of the Hayes et al (1991) paper has been published by Carlo et al (1992); a peer-reviewed scientific paper specifically critiquing another is rather unusual. An important point by Carlo et al (1992) was that a large number of associations were tested, and it is to be expected that statistical chance will result in one or a few positive associations, and that chance increases when the odds ratio is barely significant, as was the case here.

Other considerations in judging the carcinogenicity of 2,4-D are its ability to cause mutation, and its behaviour in the body. Substances that initiate the carcinogenic process directly are usually mutagens. The large number of mutagenicity assays on 2,4-D was discussed earlier in this paper. Most of the work was negative; 2,4-D is considered to have very weak mutagenic activity, if any. 2,4-D is quickly excreted and not changed in the body; most, if not all carcinogens are converted to more reactive forms in the process of detoxification. 2,4-D is not stored in any tissues. The human dosages measured in a multitude of exposure studies are very low relative to the no-effect doses in all of the experimental studies.

A conclusion that 2,4-D has little potential for carcinogenic effect is supported by the absence of effects in animal carcinogenicity assays, the absence of genetic effects of 2,4-D, the behaviour of the herbicide in the body and the biologically inconsistent and ambiguous results of epidemiology studies.

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Glossary

Active transport – Molecules within cells or in fluids outside the cells may move across membranes passively by diffusion, just as a drop of dye might spread in water, or by an energy consuming process where the molecule is moved across a membrane into another compartment where the concentration may be higher. Without this "pumping" mechanism molecules would move the wrong way. The best examples of this active transport process with respect to herbicides is the movement of organic acids like 2,4-D and triclopyr from the brain to the blood and from the blood into the kidney tubules for excretion. Glyphosate, on the other hand, moves out by diffusion without help from active transport.

Acute toxicity – (Short term toxicity) – Acute toxicity is the quality or potential of a substance to cause injury or illness from a single dose or short period of exposure. See **subacute, subchronic and chronic.**

Adjuvant – Any additive to a pesticide formulation that is not active itself, but is intended make the active ingredient work better.

Adduct – Any large biological molecule such as DNA, or proteins such as haemoglobin or albumin to which some reactive molecule has attached chemically. Adducts occur normally as a result of contact with substance that arise in the cells during metabolism, or exposure to toxic substances or their products.

Biodegradable – Capable of being metabolized by a biological process or organism.

Cancer – A malignant growth of potentially unlimited size that invades local tissues, and may spread to other parts of the body.

Carcinogen – A chemical capable of inducing cancer.

Carcinogenic – Capable of causing cancer.

Chronic toxicity – (Long-term toxicity) – Chronic toxicity is the quality or potential of A substance to cause injury or illness after repeated exposure for a long period of time. Chronic toxicity tests run for a year or more; for rodents the period may extend through the entire life span. A chronic effect persists for months or years and may arise from acute or long term exposure. See **acute, subacute, subchronic.**

Contaminant – In a formulation, usually residues or impurities from the manufacturing process present in small quantities. Contaminants must be identified to the regulatory agency, which judges whether they are of concern.

Deoxyribonucleic Acid – See **DNA**

Degradation – Breakdown of a compound by physical, chemical or biochemical processes into basic components with properties different from those of the original compound.

Detection limit. The lowest concentration of a chemical that can be identified in a substance (e.g., soil, foliage or body fluids). Analytical sensitivity varies among chemicals, and in different media. The detection limit is usually lower than the level that can be reliably measured. For example, it may be possible to find a substance present at 0.01 parts per billion, but only at levels above 0.03 ppb can the amount be stated.

Detoxication (Detoxification) – The biochemical process of changing a chemical in the body to a less toxic form or to a form that can be more easily excreted.

Dose – The amount of a chemical that actually enters the body to be distributed to all of the organs and cells. Distribution to tissues and cells is selective, and depends on the nature of the chemical and characteristics of each kind of cell.

Dose-response relationship – The central idea in toxicology and in pharmacology (which is the science dealing with beneficial effects of therapeutic drugs). As the dose (or concentration) of a chemical increases, the effect increases, and as the dose is lowered, the effect becomes less. This response pattern applies to every interaction between a chemical and a biological system, whether human, fish, bacteria or any other kind of organism or tissue. The dose-response relationship is absolutely essential to judgement of the effect of any chemical.

DNA (Deoxyribonucleic Acid) – The genetic library in each cell that contains all of the instructions for building and operating the body. Each kind of cell contains all of the information for the whole body. Only the information needed for each kind of cell is used by that cell; the rest is repressed. Liver cells do not try to be muscles, and muscles do not try to become brain cells, but they contain all of the information.

EC_{50} – Acronym for median effective concentration.

Environmental chemistry – The study of the physical, chemical and biological processes that govern behaviour and fate of a chemical such a pesticide after it is used.

Enzymes – Complex proteins that catalyze (expedite) biochemical reactions. See Metabolism.

Epidemiology – The scientific study of the cause, distribution, and control of epidemics or other disease in a region. In the context of these reports, epidemiology is the study of possible associations between environmental and occupational chemicals and occurrence of diseases. The term "associations" is used in its statistical sense, which means that the relationship cannot demonstrate cause and effect.

Exposure – Amount of a chemical that reaches a surface from which it might be absorbed. The dose is some fraction of the exposure. Exposure does not include material that is on nearby foliage or other surfaces. It is only the material that reaches the skin (by contact), respiratory tract (by inhalation) or digestive tract (by ingestion).

Foetus – The later stage of mammalian development in the womb. In human, this refers to the unborn child during the period of uterine life from the end of the second month until birth.

Foetal toxicity – Direct effects of a toxicant on the foetus, independent of effects on the mother.

Formulation – A complete pesticide preparation as sold by a manufacturer for practical use. It includes the active ingredient and any necessary adjuvants and solvents. For use, it may or may not require further dilution or mixing with other substances. Formulation can also be defined as the process used by manufacturers in preparing a pesticide for practical use.

Half-life – The length of time required for disappearance of half of the material present in an organism or in environmental media. It is a more useful idea than "persistence" because it allows prediction of the time required to reach low target levels with out making measurements over exceedingly long periods. A better term is "Half-time," because the information only relates to a given location, and says nothing about the processes that deplete the chemical. If it evaporates or is carried away intact by water it may still exist in its original form. The term "half-life" originated with description of radioactive decay, in which elements become a totally different substance. The English language sometimes loses precision as it evolves.

Hazard – The kind of effect that a chemical can cause. Cancer, liver disease, skin irritation, reproductive problems, or some other more or less specific response that can be defined and measured. The term is also used non-specifically to signify any dangerous situation.

Herbicide – A chemical substance or cultured biological organism, used to kill or suppress the growth of plants.

Hormone – A substance secreted by specialized endocrine cells and transported by the blood stream throughout the body to regulate biochemical activity of other cells. Insulin and testosterone are hormones.

Immune system – All of the structures and cells and their products that protect against infectious organisms and against cells of the body that have become altered in the very early development of cancer.

Inert ingredient – Any component of a formulation that is purposely added and does not have pesticidal activity. Includes solvents and adjuvants, not manufacturing impurities.

Irritation – A purely local or topical reaction which may include redness, blistering, swelling, burning or itching.

LD_{50} – Acronymn for Median lethal dose.

Lethal – Causing death.

Lethal concentration (LC_{50}) – Rate at which 50 percent of test animals will be killed.

LOAEL – Acronym for lowest-observed-adverse-effect level.

Lowest-observed-adverse-effect level (LOAEL) – The lowest measured amount of a chemical that produces significant increases in frequency or severity of adverse effects in exposed subjects. in the general sense it includes all biochemical, pathological, behavioral, reproductive, genetic and other measurable changes. the term may also be applied to any specific parameter under observation.

Malignant – Deadly or very injurious. As applied to cancer, invasive of local tissues and metastatic (migration of cancer cells to other tissues).

Margin of Safety – (MOS) – The difference between the estimated dose of a pesticide and the NOAEL. A **MOS** of 100 (estimated dose 100 fold less than the NOAEL) is usually considered to assure that no adverse effects will occur.

Median effective dose (ED_{50}) – The dose or dose rate that causes 50% of subjects to respond. The nature of response must be specified, ie, sedation, elevated blood pressure, death. The ED_{10} is the dose effective in 10% of animals.

Median lethal dose (LD_{50}) – The dose of a chemical, biological agent, or other substances that causes death in 50% of defined test animals.

Metabolism – The sum total of the biochemical reactions that a chemical undergoes in an organism. The processes include biochemical (enzymatic) reactions in the cells of the body that convert nutrients to energy and structural materials of the body; reactions that change wastes so they can be removed; and reactions that convert foreign substances, such as some pesticides to forms that can be excreted.

MOS – Acronym for margin of safety.

Mutagenic. Capable of producing genetic changes.

Mutagens – Chemicals that are able to induce gene or chromosome damage that is stable and survives cell division to reach the next generation of cells. See **mutation.**

Mutation – Genetic change in DNA of a cell that can be transmitted to the next generation of cells. If in sperm or egg cells, a mutation may be transmitted to offspring. If in somatic (body) cells such as liver, muscle or other organs, a mutation may pass to daughter cells in the organ. The change may have no effect on cell function or it may damage the cell, or even imaginably improve it.

NOAEL – Acronym for no-observed-adverse-effect level.

No-observed-adverse-effect level (NOAEL) – The dose rate or concentration at and below which no adverse effects can be detected. (See **threshold; SEE LOAEL)** If the estimated dose of a herbicide to a worker is very low compared to the **NOAEL** for the most sensitive effect found in the laboratory, no harmful effect is to be expected.

NOEL – Acronymn for **no-observed-effect level.**

No-observed-effect-level – (NOEL) – Dose of a chemical or biological agent at which there are no biologically or statistically significant effects attributable to treatment. The term can refer to adverse, beneficial or meaning-less effects and is falling out of use in toxicology.

Oncogenic – Able to cause cancer.

Persistence – The duration of measurable concentrations of a pesticide in soil, foliage or other media. See **Half-life.**

Pesticide – Any chemical (or biological product) intended to control or kill pests. Herbicides, insecticides, fungicides are all pesticides. The term is sometimes incorrectly used to mean only insecticide, for example "pesticides and herbicides."

Pharmacokinetic – Relating to the rate and pattern of the absorption, distribution, metabolism and excretion of drugs in an animal.

Reference dose (RfD) – Any oral dose below the RfD is considered unlikely to be associated with an adverse health effect and is therefore acceptable. The RfD is usually based on the most sensitive oral NOAEL, with all appropriate safety factors included.

Registration – The process by which government (e.g., Canadian federal government) authorities determine that a pesticide is suitable for use. Standards of public and worker safety, environmental impact, and usefulness must all be met.

RfD – Acronym for reference dose.

Risk – The probability (likelihood) that some adverse or undesirable effect will take place in the future, as a result of some specified activity. Risk may relate to health, finances or any other kind of undesirable impact. Real risk may be so small that it cannot be distinguished from zero, or so great that it is a certainty. In the context of pesticides, risk is the probability that use of the pesticide will result in some specified harmful effect on workers or the public. Risk assessment is the process of estimating that probability.

Safety Factor – See **Margin of Safety**

Sensitization – the initial exposure of an organism to specific antigen (foreign protein or chemically altered body protein) resulting in a response of the immune system such that subsequent exposure induces an allergic reaction.

Subacute – Extending over a few days to perhaps a month. This and related terms do not carry defined time periods; consequently there is overlap in the way they are used. See **Acute, subchronic and chronic.**

Subchronic – For experimental studies, relatively long term, but not as long as a chronic study. Typically three to six months. See **acute, subacute, and chronic.**

Teratogen – A chemical that can cause birth defects.

Teratogenic – Relating to or able to produce birth defects.

Threshold – The lowest dose that will produce a given effect. As a practical matter, the threshold is little different from the **NOAEL.**

Tolerance – lesser than normal sensitivity of an individual to the adverse effect of a chemical. also, the allowable residue of a pesticide on a food or feed crop.

Toxicant – a toxic agent; a poison.

Toxicity – The whole pattern of harmful effects (illness and other undesirable effects) that a chemical can cause. It is a property of the chemical; it does not change.

Toxicology – The group of scientific disciplines that identifies and studies the adverse effects of chemicals on biological systems, whether in the laboratory or in the field.

Toxin – A poisonous substance produced by a living organism. The term is sometimes incorrectly used in reference to non-biological chemicals.

Tumour – a new growth of cells multiplying progressively and without control. Classically, the term means a swelling.

APPENDIX A: Risks to workers using 2,4-D formulations: Summaries of published work

In the summaries below, the numbers in parentheses are statistical 95% confidence intervals. In other words, there is 95% certainty that the real figure lies somewhere between the two limits. The initial number is statistically considered most likely to be correct. As a rule of thumb, it is generally assumed that when the low end of the range falls below one, the figure does not represent an effect. (In some methods, the normal comparison is 100, which represents no effect. In this case if the low end is below 100 the association is considered to be weak or non-existent.) As an example, if the estimated risk is 1.4 with a confidence interval of 0.4-3.0 it probably does not represent a real effect. Because of these uncertainties, small differences, say, a 1.5 fold excess can usually be regarded only as suggestive. In addition, when there are major uncertainties about exposure or other variable information, even larger estimates of risk may not have the weight needed for decision making. Comparisons among the various studies illustrate the difficulty in finding convincing evidence of either an effect or absence of effect. In the summaries below:

NA = No association with pesticide exposure

STS = Soft tissue sarcoma

NHL = Non-hodgkin's lymphoma , and

HD = Hodgkin's disease

1. Cantor, K.P. and Booze, C.F. Jr. (1991). Mortality among aerial pesticide applicators and flight instructors: a reprint. Arch. Environ. Health 46:110–116.
Aerial applicators were compared with flight instructors, on the basis of Standard Mortality Ratios (SMR). All lymphopoietic cancers included, which may have included Hodgkin's disease. Standard Mortality Ratio of 96 for applicators compares with 54 (20-117) for instructors. For all malignancies applicators SMR was 74, and SMR for instructors was 64. Malignancies of pancreas, larynx, skin, prostate, kidney were slightly greater, others same or lower. All numbers compared with general population, normal SMR is 100. No information about specific pesticides.

2. Coggon, D., Pannett, B. and Winter, P. (1991). Mortality and incidence of cancer at four factories making phenoxy herbicides. Brit. J. Indust. Med. 48:173–178.
Four cohorts (groups) of workers in British manufacturers of phenoxy herbicides. Wide range results from very few cases, where even a single case may result in large apparent difference. The Standard Mortality Ratio for all cancers was 100 (71-138), for lung cancer 134 (81-210) and for NHL 229 (28-827). No association for STS or HD.

3. Corrao, G., Calleri, M., Carle, F., Russo, R., Bosia, S. and Piccioni, P. (1989). Cancer risk in a cohort of licensed pesticide users. Scand. J. Work Environ. Health 15:203–209.
25,945 farmers in Italy licenced to use pesticides. NHL 1.8 (1.2–2.5).

4. Green, L. (1991). A cohort mortality study of forestry workers exposed to phenoxy acid herbicides. Brit. J. Indust. Med. 48:234–238.
Right-of-way vegetation management, multiple herbicides, mostly phenoxies. NA, STS and NHL.

5. Hardell, L. and Eriksson, M. (1988) . The association between soft tissue sarcomas and exposure to phenoxyacetic acids. A new case-referent study. Cancer 62:652–656.
Two analyses (a) Exposure estimated by interview. STS 3.3 (1.4-8.1) (b) All occupations in agriculture and forestry. NA. Some diagnoses of STS questionable. Adjustments could change risk ratio to, variously, 2.2 on the low side to 4.1 on upper side.

6. Hardell, L., Eriksson, M. and Degerman, A. (1994). Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Research 54:2386–2389.
Same population as 5, above. NHL, 5.5 (2.7-11). No association with occupation, including forestry and farming.

7. Hoar, S.K, Blair, A, Holmes, F.F., Boysen, C.D., Robel, R.J., Hoover, R. and Fraumeni, J.F., Jr. (1986). Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma. J. Am. Med. Assoc. 256:1141–1147.
(a) All users of herbicides. NHL 1.6 (0.9-2.6) STS and HD, NA
(b) Used herbicides more than 20 days per year. NHL 6.0 (1.9-19.5) STS and HD, NA
(c) Applied herbicides self, often. NHL 8.0 (2.3-27.9) NHL and HD, NA Estimated risk decreased with reported use of protective clothing, increased with use of backpack equipment. This study found risk associated with use prior to 1945, before 2,4-D was ever used. This has been the most widely quoted study of association between herbicides, including 2,4-D. Reviews uniformly state the study well done in spite of the finding of risk before use began, and almost uniformly conclude that this evidence is not sufficient to support a causal hypothesis.

8. Lynge, E. (1993). Cancer in phenoxy herbicide manufacturing workers in Denmark, 1947-87 – and update. Cancer causes and Control 4:261–272.
Cancer in phenoxy manufacturing personnel in Denmark. 4400 workers during period 1947-1987. STS 2.3 (0.6-5.8) NHL, NA. Overall cancer incidence same as Danish population.

9. Pearce, N. (1989). Phenoxy herbicides and non-Hodgkin's lymphoma in New Zealand: frequency and duration of herbicide use. Brit. J. Indust. Med. 46:143–144.
(a) Duration of use more than 15 years, NHL (0.6-2.3). (b) 10-19 days use per year, NHL 2.2 (0.4-12.6). (c) More than 20 days use per year, NHL 1.1 (0.3-4.1).

10. Smith, J.G. and Christophers, A.J. (1992). Phenoxy herbicides and chlorophenols: A case control study on soft tissue sarcoma and malignant lymphoma. Brit. J. Cancer 65:442–448.
(a) risk for exposure of at least one day, STS 1.0 (0.3-3.1), NHL 1.5(0.6-3.7) (includes HD) . (b) risk for exposure more than 30 days, STS 2.3(0.5-8.0), NHL 2.7(0.7-9.6) Hodgkins and non-Hodgkins lymphomas collectively; 14 types identified. 16 types of soft tissue sarcoma identified. Risks stated not greater than unity.

11. Vineas, P., Terracini, B., Ciccone, G., Cignetti, A., Colombo, E., Donna, A., Maffi, L., Pisa, R., Ricci, P., Zanini, E. and Comba, P. (1986). Phenoxy herbicides and soft tissue sarcomas in female rice weeders. A population-based case-referent study. Scand. J. Work, Environ. Health 13:9–17.
Female rice weeders in Italy. STS 2.7(0.59-12.37) Paper notes the wide variety of cancer types under STS. Authors suggest that this study underestimates risk.

12. Vineas, P., Faggiano, F., Tedeschi, M. and Ciccone G. (1991) Incidence rates of lymphomas and soft tissue sarcomas and environmental measurements of phenoxy herbicides. J. Nat. Cancer Inst. 83:362-363.
Community with extensive use of phenoxy herbicides. Exposure estimated on basis of contamination of surface water.
(a) Males. STS 1.8(1.2-2.6), NHL, 8.8(7.4-10.4), HD 3.3(2.4-4.4
(b) Females. STS 0.9(0.5-1.4), NHL 5.8(4.7-7.0), HD 1.5(0.9-2.3

13. Wigle, D.T., Semenciw, R.M., Wilkins, K., Riedel, D., Ritter, L., Morrison, H.I. and Mao, Y. (1990). Mortality study of Canadian male farm operators: Non-Hodgkin's lymphoma mortality and agricultural practicies in Saskatchewan. J. Nat. Cancer Inst.
82:575–582.
(a) 70,000 Saskatchewan farmers as identified in

1971 Census of Agriculture, NHL 0.92(0.8-4.0) (b) Farmers reporting use of herbicides compared with those reporting no use, for 100-249 acres, 1.47(0.94-2.41) (c) for 250 + acres, 1.34(0.74-2.38). There was also an association with expenditures of fuel and on pesticides.

14. Wiklund, K., Lindefors, B-M. and Holm, L-E. (1988a). Risk of malignant lymphoma in Swedish agricultural and forestry workers. Brit. J. Indust. Med. 45:19–24.
354,620 men who were employed in agriculture or forestry in 1960.
(a) Entire group, NHL, NA; HD 2.26(1.25-3.69).
(b) silviculture, NHL, NA; HD 4.45(1.41-10.15). (c) Fur farming, NHL NA; HD 2.92(0.79-7.41. NHL risk lowest in forestry.

15. Wiklund, K., Dich, J. and Holm, L-E. (1988b). Soft tissue sarcoma risk in Swedish licensed pesticide applicators. J. Occup. Med. 30:801–804.
20,245 applicators, all pesticides.
(a) Entire group, STS 0.9(0.4-1.9)
(b) Five year latency STS 1.0(0.4-2.2)
(c) Ten year latency 1.0(0.3-2.7) Latency refers to period since first exposure. The longer the latency, the greater the presumed risk.

16. Wiklund, K., Dich, J., Holm, L-E. and Eklund, G. (1989). Risk of cancer in pesticide applicators in Swedish agriculture. Brit. J. Indust. Med. 46:809-814.Same cohort as Wiklund et al., 1988b. NHL 1.01(0.63–1.54).
HD 1.2(0.60-2.16). Total cancer risk 0.86(0.79-0.93. Testicular cancer increased with years since licence, maximum risk 2.54(1.1-5.0)

17. Woods, J.S., Polissar, L., Severson, R.K., Heuser, L.S. and Kulander, B.G. (1987). Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in western Washington. J. Nat. Cancer Inst. 78:899–910.
Case-control, 128 STS and 576 NHL cases, Washington State. (a) Any past occupational exposure to phenoxy herbicides, STS 0.8(0.5-1.2); NHL 1.07(0.8-1.4). (b) Men who had been farmers, NHL 1.33(1.03-1.7). (c) Forest herbicide applicators, NHL 4.8(1.2-19.4) Small association with phenoxies plus other chem; no link to any specific product. Association of NHL only if use period was 15 years or more and at least 15 years elapsed since last use.

18. Zahm, S.H., Weisenburger, D.D., Babbit, P.A., Saal, R.C., Vaught, J.B., Cantor, K.P. and Blair, A. (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349–356.
Eastern Nebraska farmers.
(a) Mixed or applied 2,4-D, NHL 1.5(0.9-2.5)
(b) on farm where 2,4-D was used, but did not handle, NHL 1.2(0.3-4.2)
(c) 6-15 years of use NHL 2.8(1.1-7.1)
(d) 16-20 years of use, NHL 0.6(0.1-2.1).
No excess NHL associated with ever living or working on a farm. Note the apparent decreased risk with longer period of use.

APPENDIX B: Contaminants and inert ingredients

Contaminants are unnecessary substances in the technical material or in the formulation. Usually they are unreacted materials remaining from manufacture of the product that have been considered unimportant and left in the finished product because there was no compelling reason to take them out. They may also be products of side reactions that occur in manufacture. They are in fact often unimportant, but not always, and they represent a proper area of concern that is only recently being addressed.

Inert ingredients are materials that have no pesticidal activity, but are added deliberately to the formulation for some purpose. They may be solvents, surfactants, preservatives, dyes or other substances that contribute to the utility of the formulation. It has always been assumed that inert ingredients do not represent a risk; traditionally their nature has been kept confidential by manufacturers because of presumably unique contributions to the quality of the finished formulation. Regulatory agencies and the public are beginning to demand identification of "inerts," some of which are known in other contexts to possess significant toxicity. In forestry herbicides, the most toxicologically significant inert ingredients are the kerosene or diesel oil used as solvents in some ester formulations.

Some attention has been focused on surface-active substances that are used in many formulations. Because surfactants are virtually ubiquitous in modern life, there has been relatively little concern about possible adverse effects. Such substances are found in multitudes of personal care products, almost every household cleaning preparation and in numerous industrial processes. Garry et al. (1999) observed the occurrence of nuclear fragments in cultured lymphocytes exposed to herbicides and certain adjuvants, in *vitro*. There was little response among the herbicide formulations but the four adjuvants tested produced dose-related increases. The authors comment on the need to further define possible effects. In another study the same group found evidence as well of a relation between high 2,4-D levels and a reversible instability of one region of the genome (Garry et al., 2001).

In 2,4-D, there have been three potential contaminants of concern. In spite of considerable publicity, none has represented a health threat. In earlier production, there were measurable quantities of 2,4-dichlorophenol, one of the starting materials in manufacture. It was found to have some ability, apparently through severe irritation, to promote skin tumours that had already been started in mice by direct carcinogens. The doses required were so large as to cause considerable other damage and death in the mice, and were too large to be achieved even experimentally by administration of contaminated 2,4-D. The effect was never considered to represent a risk to be associated with 2,4-D, but the process was changed to eliminate dichlorophenol from the final product. Canadian standards for 2,4-D include a limit of 0.3 % total free phenol.

Earlier production sometimes was found to contain N-nitroso-dimethyl amine, a known carcinogen. It arose in some cases from use of nitrite as a rust inhibitor in the formulation, and it is possible that the same contaminant may have occurred in the manufacture of the amine salts of 2,4-D. The amounts found were not considered by regulatory agencies to represent a significant risk, but they too have been eliminated from formulations.

The third group of contaminants is of greatest potential concern, but in practical terms does not represent an appreciable risk. In 1980 analysts at Agriculture Canada found several chlorodibenzo-p-dioxins in 2,4-D (Cochrane et al., 1981). No 2,3,7,8-TCDD was found, and the chlorodioxins that were found were not among those considered to represent a health threat.

Nevertheless, USEPA undertook a search for chlorodioxins in more than 30 samples of 2,4-D, finding no 2,3,7,8-TCDD and traces of some others, including 2,7-dichlorodibenzo(p)dioxin (2,7-DCDD). 2,7-DCDD is the isomer that should be expected to predominate in 2,4-D, and it was found in three of the samples at low levels. Even if it were assumed that all 2,4-D is contaminated at levels higher than were found in the few positive samples, 2,7 DCDD represents no health concern. (It is of interest that Norstrom et al (1977) assayed several samples of 2,4-D manufactured between the late fifties and 1965, and found no chlorodioxins at a detection limit of 50 parts per billion. One sample had a trace of a tetrachloro-dibenzo furan.

There is no current published data on chlorodioxin contaminants in 2,4-D. Under the Pest Control Products Act, Agriculture Canada has specified standards for 2,4-D contaminants. The upper limit is 30 parts per billion parts 2,4-D (PPB), with maxima for the following isomers:

monochlorodibenzo-p-dioxin	10 ppb
2,7-dichlorodibenzo-p-dioxin	10 ppb
1,3,7-trichlorodibenzo-p-dioxin	10 ppb
1,3,6,8/1,3,7,9-tetrachlorodibenzo-p-dioxin	10 ppb
2,3,7,8-tetrachlorodibenzo-p-dioxin	N.D.

N.D.: non-detectable at 1 ppb

Typical production in the mid-nineties contained about half the levels noted.

In 1987 the U.S. Environmental Protection Agency issued a data call-in for each manufacturer, listing the required analytical sensitivity for each of 15 dioxin and dibenzofuran species. Those data were provided and there has been no response by the regulatory agency. It follows that any contaminants of this class were not found or were found at levels that did not trigger health protective action by the agency.

Titles in this Series

1 Principles of health effects evaluation and risk estimation for chemicals that may be encountered in forest vegetation management

2 Pesticide testing for registration: toxicity, environmental behaviour, and epidemiology

3 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part I: Risk to workers associated with exposure to emissions from power saws

4 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part II: Exposure to and absorption of herbicides used in forestry

5 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part III: Risk to workers using 2,4-D formulations

6 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part IV: Risk to workers using glyphosate formulations (e.g., Vision®, Roundup®, Vantage Forestry® and Forza®)

7 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part V: Risk to workers using hexazinone formulations (Pronone®, Velpar® L)

8 Toxicology and potential health risk of chemicals that may be encountered by forest vegetation management workers. Part VI: Risk to workers using triclopyr formulations (Release®, or Garlon 4®)

9 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options: Summary

Title Number

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